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Bioactive Constituents, Metabolites, and Functions

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C30 and C31 Triterpenoids and Triterpene Sugar Esters with Cytotoxic Activities from Edible Mushroom *Fomitopsis pinicola* (Sw. Ex Fr.) Krast

Xing-Rong Peng,[†] Hai-Guo Su,[†,‡] Jun-Hong Liu,[†,‡] Yan-Jie Huang,[†,‡] Xin-Zhi Yang,[†,‡] Zhong-Rong Li,[†] Lin Zhou,[†] and Ming-Hua Qiu*[,†]

[†]State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Science, Kunming 650201, People's Republic of China

[‡]University of the Chinese Academy of Science, Beijing 100049, People's Republic of China

Abstract: *Fomitopsis pinicola* (Sw. Ex Fr.) Krast has been commonly used as a health food source and anti-tumor agent. To uncover bioactive key composition of *F. pinicola*, in our study, we investigated the chemical constituents of methanol extract of *F. pinicola* and thirty-five lanostane-type tritetpenoids, including thirteen new compounds (**1**–**13**) and twenty-two known analogues (**14**–**35**) were isolated. Among them, compounds **1**–**9** were C30 lanostane triterpenoids and triterpene sugar esters, while compounds **10**–**13** were C31 triterpenoids and triterpene sugar esters. Their structures and absolute configurations were elucidated by extensive 1D, 2D NMR, MS and IR spectra. Furthermore, cytotoxic activities of all isolates against five human tumor cell lines (HL-60, A549, SMMC-7721, MCF-7 and SW480) were evaluated. The results showed that compounds **12**, **14**, **17**, **18**, **22** and **23** displayed cytotoxic effects against five human tumor cell lines with IC_{50} values ranging from 3.92–28.51 μM. Meanwhile, compounds **9** and **35** exhibited selected inhibitory activities against HL-60, SMMC-7721 and MCF-7 with the IC_{50} values in the range of 13.57–36.01 μM. Furthermore, the flow cytometry analysis revealed that compounds **17**, **22** and **35** induced apoptosis in HL-60 cell lines. Their structure-activity relationships were preliminarily reported. These findings indicate the vital role of triterpenoids and their glycosides in explaining anti-tumor effects of *F. pinicola* and provide an important evident for the further development and utilization of this fungus.

Keywords: *Polyporaceae, Fomitopsis pinicola, C30 triterpenoids, C31 triterpenoids, cytotoxic activity*

INTRODUCTION

Recent years, mushroom production has substantially increased. One of reason is that many mushrooms are health food source due to their plentiful protein, vitamins and minerals, as well as little fat.[1] Another one is that some edible mushroom species, such as *Ganoderma* (*G. lucidum* and *G. sinense*),[2,3] *Tricholoma* (*T. matsutake*),[4] *Stropharia* (*S. rugosoannulata*),[5] and *Pleurotus* (*P. eryngii*),[6] have significant pharmacological attributs.[7]

Fomitopsis pinicola (Sw. Ex Fr.) Krast is belonging to Basidiomycetes fungus and has been widely used as health food and medicinal mushroom in Asia.[8] The extract of *F. pinicola* was reported to exhibit antioxidant, antitumor *in vitro* and *in vivo*[9] and hypoglycemic activities.[1] Except for C30 lanostane triterpenoids and their glycosides, C31 triterpenoids and their glycosides were one of the main constituents of *F. pinicola*, and these components displayed anti-bacterial,[10] anti-microbal effects[11] and inhibition of COX-1 and COX-2.[12] Notably, lanostane-type triterpenoids showed more potent anti-tumor activity in recent years.[7] Sun et al[13] found that the tumor-inhibition rate of 3-acetoxyl-lanotane-8,24-dien-21 oic acid was 67.16% at a dose of 10 mg/kg/d. Furthermore, Bao et al[14] confirmed that this compound can improve immunity of rats. Polyporenic acid B with a C31 skeleton showed cytotoxicity against the HCT116, A549 and HepG2 cell lines (IC_{50} values: 8.4, 12.1 and 12.2 μM).[15] Lanostane triterpenoids from *Tricholoma pardinum*, pardinols B and E–H showed inhibitory activities against different tumor cell lines to some content.[16]

Thus, to find out more triterpenoids with cytotoxicity, a systematic

phytochemical investigation of the fruiting bodies of *F. pinicola* was carried out. Our efforts led to the isolation of thirty-five lanostane triterpenoids and triterpene sugar esters, including nine new C30 triterpenoids (**1**–**9**), four new C31 triterpenoids (**10**–**13**) and twenty-two known compounds (**14**–**35**). Furthermore, their cytotoxic activities against HL-60, A549, SMMC-7721, MCF-7 and SW480 cell lines were evaluated, and apoptosis assay of bioactive compounds were tested. Meanwhile, some preliminary structure-cytotoxicity relationships were described.

MATERIALS AND METHODS

General Experimental Procedures. Optical rotations were obtained with a Jasco P-1020 polarimeter (Jasco, Tokyo, Japan). UV spectra were recorded using a Shimadzu UV2401PC spectrophotometres (Shimadzu, Kyoto, Japan). ^{1}H and ^{13}C NMR spectra were measured on Bruker AV-400 and AV-600 instruments (Bruker, Zurich, Switzerland) using Transcranial magnetic stimulation (TMS). ESIMS and HRTOF-ESIMS data were recorded on an API QSTAR Pulsar spectrometer (Waters, UK) and infrared spectra were recorded on a Bruker Tensor-27 instrument by using KBr pellets (Bruker, German). Circular dichroism spectra were taken on an Applied Photophysics spectropolarimeter (Agilent, USA). Semi-preparative HPLC was performed on an agilent 1100 series instrument (Agilent, Technologies, Foster City, CA, USA) with a Agilent ZORBAX C18 (5 μm, 9.6 mm × 250 mm) and a Thermo Hypersil GOLD Phenyl (5 μm, 10 mm × 250 mm). TLC was performed on precoated TLC plates (200-250 μm thickness, F254 Si gel 60, Qingdao Marine Chemical, Inc.) with compounds visualized by spraying the dried plates with 10% aqueous H_2SO_4

followed by heating until dryness. Silica gel (200-300) mesh, Qingdao Marine Chemical, Inc.), Lichroprep RP-18 (40–63 μm, Merck) and Sephadex LH-20 (20–150 μm, Pharmacia) were used for column chromatography. Methanol, trichloromethane, ethyl acetate, acetone and *n*-buthanol were purchased from Tianjing Chemical Reagents Co. (Tianjing, China).

Plant Materials. The fruiting bodies of *Fomitopsis pinicola* (Sw. Ex Fr.) Krast [MB#101927] (http://www.mycobank.org/quicksearch.aspx) were purchased from Luosiwan Chinese medicine market of Kunming in Yunnan Province, PR China, in July 2017. The specimen (2017fp07) was kept in Kunming Institue of Botany. This fungus was identifed by Prof. Yang Zhuliang, who is a fungal taxomonix and is working in Kunming Institute of Botany.

Extraction and Isolation. The fruiting bodies of *F. pinicola* (30 kg) were extracted with 95% MeOH/H_2O (45 L) under reflux three times at 60℃, each for 3 hours. The combined methanol extracts were evaporated under reduced pressure. The residue (3 kg) was suspended in H_2O (15 L) and extracted with petroleum ether (3 × 15 L, PE) and ethyl acetate (3 × 15 L, EtOAc, EA). The volume of the combined EA extracts (1.5 kg) was reduced to one-third under vacuum. The residue was analyzed by UPLC-DAD/MS. Combined the maximum UV absorption bands and range of molecula weight indicated that this part contained triterpenoids (Supporting Information, Figure SA). Then, EA part was fractioned using macrosporous resin (D-101, MeOH–H_2O = 20:80, 40:60, 70:30, 90:10, 100:0, 100 L) to give five fractions: fractions I–V. Fraction IV (100 g) was treated by silica gel column

chromatograph (CC) (10 L, $CHCl_3$–MeOH = 80:1, 50:1, 20:1, 5:1) to give four sub-fraction (Fr. IV-1→Fr. IV-4). Fr. IV-2 (51 g) was subjected to silica gel CC ($CHCl_3$:Acetone = 50:1, 20:1, 10 L) to obtain six sub-fractions (Fr. IV-2-1→Fr. IV-2-6). After Fr. IV-2-1 (325 mg) was treated by silica gel CC ($CHCl_3$–Acetone = 50:1), compound **4** (50 mg) and piptolinic acid D (**24**, 12 mg) were obtained. Fr. IV-2-2 (132 mg) was separated on the silica gel CC eluted with $CHCl_3$:MeOH = 50:1 (v/v, 5 L) to obtain three subfractions (Fr. IV-2-2a→Fr. IV-2-2c). Then, Fr. IV-2-2b was further purified by semi-preparative HPLC (MeCN:H_2O:CF_3COOH = 65:35:0.1%, 30 min) with an Agilent ZORBAX C18 column and compounds **5** (12 mg, t_R = 13.2 min), **29** (10 mg, t_R = 15.3 min) and **33** (8 mg, t_R = 24.2 min) were yielded. Fr. IV-2-3 (156 mg) was applied to LH-20 using MeOH as elutes to give three subfractions. Among them, compound **10** (21 mg, t_R = 16.3 min) was purified from Fr. IV-2-3b (82 mg) by semi-preparative HPLC (MeCN:H_2O:CF_3COOH = 68:32:0.1%) with a Thermo Hypersil GOLD Phenyl column.

Fr. IV-2-4 (10 g) was separated by silica gel CC eluted with $CHCl_3$:Acetone = 50:1, 20:1, 10:1 and 5:1 to obtain four subfractions: Fr. IV-2-4-1→Fr. IV-2-4-4. Separation of Fr. IV-2-4-1 (102 mg) through semi-preparative HPLC (MeCN:H_2O:CF_3COOH = 73:27:0.1%, 35 min) with an Agilent ZORBAX C18 column to give compounds **6** (8 mg, t_R = 20.8 min), **14** (5 mg, t_R = 22.6 min), **21** (14 mg, t_R = 24.2 min) and **32** (3 mg, t_R = 33.3 min). Fr. IV-2-4-2 (387 mg) was subjected to silica gel CC using $CHCl_3$:Acetone = 30:1 (5 L) as elutes, and subsequently, Fr. IV-2-4-2b was isolated by preparative TLC (P-TLC) ($CHCl_3$:Acetone = 12:1, 80 mL)

to yield compounds **27** (12 mg), **30** (6 mg) and **31** (3 mg).

Fr. IV-2-5 (8.5 g) was applied to a silica gel column using $CHCl_3$:MeOH = 70:1 as elutes to obtain four subfracitons including Fr. IV-2-5-1, Fr. IV-2-5-2, Fr. IV-2-5-3 and Fr. IV-2-5-4. Compounds **11** (10 mg, t_R = 17.9 min), **2** (16 mg, t_R = 20.6 min), and **3** (26 mg, t_R = 35.6 min) were separated from Fr. IV-2-5-1 (92 mg) by semi-preparative HPLC (0→25 min, MeCN:H_2O:CF_3COOH = 65:35:0.1%→70:30:0.1%; 25→35 min, 70:30:0.1%; 35.1 min, 65:35:0.1%; 35.1→37 min, 65:35:0.1%) with a Thermo Hypersil GOLD Phenyl column. Fr. IV-2-5-2 (125 mg) was also treated by semi-preparative HPLC (MeCN:H_2O:CF_3COOH = 65:35:0.1%, 20 min) with a Thermo Phenyl column to give compounds **25** (10 mg, t_R = 15.7 min) and **16** (3.2 mg, t_R = 16.9 min). In addition, Fr. IV-2-5-3 (96 mg) were separated on semi-preparative HPLC eluted with MeCN:H_2O:CF_3COOH = 65:35:0.1% (32 min) with a Thermo Hypersil GOLD Phenyl column to obtain compounds **26** (12 mg, t_R = 21.3 min) and **34** (6 mg, t_R = 30.1 min).

Fr. IV-2-6 (30 g) was subjected to RP-18 CC (MeOH:H_2O = 40:60→100:0, v/v, 10 L) to give ten subfractions (Fr. IV-2-6-1→Fr. IV-2-6-10). Fr. IV-2-6-1 and Fr. IV-2-6-2 were isolated by P-TLC ($CHCl_3$:MeOH = 20:1, v/v, 80 mL) to yield compound **17** (7.4 mg) and the mixture of compounds **7** and **8**, respectively. Furthermore, compounds **7** (11 mg, t_R = 8.7 min) and **8** (10 mg, t_R = 12.3 min) were purified by chiral column (AD-H, CHIRALCEL AD-H, 5 μm, 4.6 mm × 250 mm) eluted with *n*-hexane:isopropanol = 88:12 (v/v, 13 min). Fr. IV-2-6-5 (210 mg) was subjected to silica gel CC using $CHCl_3$:Acetone = 50:1 (v/v, 10 L) as elutes and five

141 subfractions (Fr. IV-2-6-5a→Fr. IV-2-6-5e) were obtained. Subsequently, Fr.
142 IV-2-6-5a (82 mg) was treated by semi-preparative HPLC (Thermo Hypersil GOLD
143 Phenyl column) eluting with MeCN:H_2O:CF_3COOH = 65:35:0.1% (v/v, 32 min) and
144 compounds **22** (5.3 mg, t_R = 25.6 min) and **1** (50 mg, t_R = 30.6 min) were isolated.
145 Similarly, separation of Fr. IV-2-6-5b (121 mg) through semi-preparative HPLC
146 (MeCN:H_2O:CF_3COOH = 62:38:0.1%, v/v, 30 min) with a Thermo Hypersil GOLD
147 Phenyl column yield compounds **23** (3.5 mg, t_R = 14.9 min), **12** (4.2 mg, t_R = 20.6
148 min) and **15** (3.2 mg, t_R = 24.2 min). Fr. IV-2-6-6 (1.2 g) was separated by silica gel
149 CC ($CHCl_3$:Acetone = 20:1, v/v, 5 L) to give four subfractions, of which Fr. IV-2-6a
150 (45 mg) was further treated by P-TLC ($CHCl_3$:MeOH = 20:1, v/v, 100 mL) to obtain
151 compound **13** (16 mg). Fr. IV-2-6-6b (53 mg) and Fr. IV-2-6-6c (43 mg) were also
152 subjected to P-TLC ($CHCl_3$:MeOH = 20:1, v/v, 100 mL) to yield compounds **19** (2.1
153 mg) and **28** (3.2 mg), respectively. Fr. IV-2-6d (105 mg) was separated by HPLC
154 (MeCN:H_2O:CF_3COOH = 65:35:0.1%, v/v, 22 min) to obtain **9** (4.3 mg, t_R = 10.6
155 min), **35** (5.3 mg, t_R = 18.2) and a mixture (29 mg, t_R = 23.5 min). The mixture was
156 purified by P-TLC ($CHCl_3$:MeOH = 15:1, v/v, 80 mL) to yield **20** (5.2 mg) and **18**
157 (9.1 mg). The isolated procedure is shown in Scheme S1 (Supporting Information).

158 *Formipinic acid A (**1**):* White powder; $[\alpha]^{19.7}_D$ = –12.31 (c 0.16, MeOH); UV
159 (MeOH) λ_{max} (log ε): 196 (4.13) nm; IR (KBr) ν_{max}: 3424, 2935, 1461, 1220 cm^{-1};
160 ^1H and ^{13}C NMR data, Table 1; HRESIMS *m/z* 599.3976 $[M - H]^-$ (calcd for
161 $C_{36}H_{55}O_7$, 599.3953).

162 *Formipinic acid B (**2**):* White powder; $[\alpha]^{19.4}_D$ = –20.63 (c 0.11, MeOH); UV

(MeOH) λ_{max} (log ε): 196 (3.93), 246 (3.56), 251 (3.56) nm; IR (KBr) ν_{max}: 3431, 2952, 2877, 1705, 1376, 1203 cm^{-1}; ^{1}H and ^{13}C NMR data, Table 1; HRESIMS *m/z* 629.4073 $[M - H]^-$ (calcd for $C_{37}H_{57}O_8$, 629.4059).

*Formipinol (**3**):* White powder; $[\alpha]^{19.7}_D$ = –22.54 (c 0.10, MeOH); UV (MeOH) λ_{max} (log ε): 196 (3.99), 252 (3.45) nm; IR (KBr) ν_{max}: 3435, 2946, 2879, 1726, 1378, 1205 cm^{-1}; ^{1}H and ^{13}C NMR data, Table 1; HRESIMS *m/z* 661.4338 $[M + COOH]^-$ (calcd for $C_{38}H_{58}O_9$, 661.4321).

*Formipiniate (**4**):* White powder; $[\alpha]^{19.7}_D$ = –7.79 (c 0.23, MeOH); UV (MeOH) λ_{max} (log ε): 196 (4.11), 242 (3.17) nm; IR (KBr) ν_{max}: 3413, 2949, 1732, 1438, 1271 cm^{-1}; ^{1}H and ^{13}C NMR data, Table 1; HRESIMS *m/z* 715.4452 $[M - H]^-$ (calcd for $C_{41}H_{63}O_{10}$, 715.4427).

*Formipinic acid C (**5**):* White powder; $[\alpha]^{19.5}_D$ = –20.40 (c 0.10, MeOH); UV (MeOH) λ_{max} (log ε): 196 (4.28), 242 (3.54) nm; IR (KBr) ν_{max}: 3440, 2977, 1705, 1626, 1425, 1265 cm^{-1}; ^{1}H and ^{13}C NMR data, Table 1; HRESIMS *m/z* 513.3586 $[M - H]^-$ (calcd for $C_{32}H_{49}O_5$, 513.3585).

*Formipinic acid D (**6**):* White powder; $[\alpha]^{19.5}_D$ = –73.95 (c 0.13, MeOH); UV (MeOH) λ_{max} (log ε): 196 (3.97), 242 (3.40) nm; IR (KBr) ν_{max} 3439, 2967, 1735, 1636, 1415, 1246 cm^{-1}; ^{1}H and ^{13}C NMR data, Table 1; HRESIMS *m/z* 513.3578 $[M + H]^+$ (calcd for $C_{32}H_{49}O_5$, 513.3575).

*Formipinic acid E (**7**):* White powder; $[\alpha]^{19.8}_D$ = +29.44 (c 0.13, MeOH); UV (MeOH) λ_{max} (log ε): 196 (4.16), 252 (3.37) nm; IR (KBr) ν_{max}: 3438, 2965, 1735, 1382, 1187 cm^{-1}; ^{1}H and ^{13}C NMR data, Table 2; HRESIMS *m/z* 483.3133 $[M - H]^-$

(calcd for $C_{30}H_{43}O_5$, 483.3116).

*Formipinic acid F (**8**):* White powder; $[\alpha]^{19.6}_D$ = +17.71 (c 0.17, MeOH); UV (MeOH) λ_{max} (log ε): 196 (4.05), 243 (4.14) nm; IR (KBr) ν_{max}: 3438, 2968, 1736, 1705, 1452, 1461, 1228 cm^{-1}; ^1H and ^{13}C NMR data, Table 2; HRESIMS *m/z* 481.2972 $[M - H]^-$ (calcd for $C_{30}H_{41}O_5$, 481.2959).

*Formipinioside (**9**):* White powder; $[\alpha]^{19.7}_D$ = +15.19 (c 0.13, MeOH); UV (MeOH) λ_{max} (log ε): 196 (4.20), 242 (3.43) nm; IR (KBr) ν_{max}: 3432, 2955, 1737, 1635, 1383, 1074 cm^{-1}; ^1H and ^{13}C NMR data, Table 2; HRESIMS *m/z* 669.3984 $[M + Na]^+$ (calcd for $C_{37}H_{58}O_9Na$, 669.3973).

*Forpinic acid A (**10**):* White powder; $[\alpha]^{19.3}_D$ = –23.52 (c 0.12, MeOH); UV (MeOH) λ_{max} (log ε): 195 (3.80), 253 (3.43) nm; IR (KBr) ν_{max}: 3449, 2963, 1729, 1638, 1384 cm^{-1}; ^1H and ^{13}C NMR data, Table 2; HRESIMS *m/z* 685.4341 $[M - H]^-$ (calcd for $C_{40}H_{61}O_9$, 685.4321).

*Forpinic acid B (**11**):* White powder; $[\alpha]^{19.8}_D$ = –25.45 (c 0.12, MeOH); UV (MeOH) λ_{max} (log ε): 196 (3.89), 252 (3.51) nm; IR (KBr) ν_{max}: 3438, 2961, 1682, 1383, 1206, 1140 cm^{-1}; ^1H and ^{13}C NMR data, Table 2; HRESIMS *m/z* 643.4235 $[M - H]^-$ (calcd for $C_{38}H_{59}O_8$, 643.4215).

*Forpinioside (**12**):* White powder; $[\alpha]^{19.7}_D$ = –17.39 (c 0.17, MeOH); UV (MeOH) λ_{max} (log ε): 196 (4.06), 244 (3.25) nm; IR (KBr) ν_{max}: 3426, 2959, 1682, 1382, 1207, 1140 cm^{-1}; ^1H and ^{13}C NMR data, Table 2; HRESIMS *m/z* 805.4770 $[M + COOH]^-$ (calcd for $C_{44}H_{69}O_{13}$, 805.4744).

*Forpinic acid C (**13**):* White powder; $[\alpha]^{19.5}_D$ = –40.82 (c 0.17, MeOH); UV

(MeOH) λ_{max} (log ε): 196 (3.94), 251 (3.89) nm; IR (KBr) ν_{max}: 3438, 2967, 1709, 1655, 1383, 1141 cm^{-1}; ^{1}H and ^{13}C NMR data, Table 2; HRESIMS *m/z* 497.3258 [M – H]$^-$ (calcd for $C_{31}H_{45}O_5$, 497.3272).

Acid Hydrolysis of Formipinioside (9) and Forpinioside (12). The acid hydrolysis of compounds **9** and **12** were carried out using a previous described method.[12] The structural identification and configuration of the sugar were determined by HPLC method, as reported in literature.[12] By comparison with an authentic sugar, the sugar obtained from compounds **9** and **12** gave the peak of D-(+)-glycose and D-(+)-xylose at 20.7 and 9.10 min, respectively.

Cytotoxicity Assay. MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxy phenyl)-2-(4-sulfopheny)-2H-tetrazolium method was used to test cytotoxic activity.[17] All the isolates were dissolved with DMSO and then diluted to five different concentrations (40, 8, 1.6, 0.32 and 0.064 μM). Five tumor cell lines: HL-60 (leukemia cell line), A549 (lung carcinoma cell line), SMMC-7721 (hepatoma carcinoma cell line), MCF-7 (breast carcinoma cell line), and SW480 (colon cancer cell line) were respectively cultured in 96-well plates with initial density of 5000 cells/well 12 h before treatment. Then, tested compounds and positive controls having different concentrations were added to above 96-well plates for 48 h. Paclitaxol (Sigma, USA) and DDP (*cis*-platinum; Sigma, USA) were positive controls. Inhibition rates of cell proliferation after compounds treatment were determined by MTS method and IC_{50} values were calculated using Reed and Muench method.[18]

Apoptosis Assay. Apoptosis was assayed by annexin V/7-AAD double stain

followed by flow cytometry. Briefly, HL-60 cells were exposed to 20 μM tested compounds, 0.1% DMSO (negative control) or 0.1 and 10 μM doxorubicin (positive control) for 24 h. Following incubation, cells were collected, washed with annexin-binding buffer, and stanined with annexin V-phycoerythrin (PE) and 7-amino-actinomycin (7-ADD). After a 30 min incubation at 15 °C in the dark, apoptosis induction was determined by flow cytometry (BD FACSCalibur).

RESULTS AND DISCUSSION

Thirty-five lanostane triterpenoids and triterpene sugar esters including thirteen new compounds (**1**–**13**) and twenty-two known analogues (**14**–**35**) were isolated from *F. pinocola*. Twenty-two known lanostane triterpenoids were identified to be fomiroid A (**14**),[19] 15α-hydroxy-3-oxolanosta-8,24-dien-21-oic acid (**15**),[20] pinicolic acid E (**16**),[21] ganosinoside A (**17**),[22] fomitoside C (**18**),[12] 15α-hydroxytrametenolic acid (**19**),[23] fomitoside I (**20**),[12] 3β-acetoxy-15α-hydroxylanosta-8,24-dien-21-oic acid (**21**),[20] 3α-acetoxy-5α-lanosta-8,24-dien-21-oic acid ester β-D-glucoside (**22**),[24] fomitoside H (**23**),[12] piptolinic acid D (**24**),[25] 16α-hydroxy-3-oxolanosta-7,9(11),24-trien-21-oic acid (**25**),[26] 3β-hydroxy-16-oxolanosta-7,9(11),24-trien-21-oic acid (**26**),[27] 3β-*O*-acetyl-16α-hydroxydehydrotrametenolic acid (**27**),[28] 3β,16α-dihydroxylanosta-7,9(11),24-trien-21-oic acid (**28**),[29] 3-*epi*-dehydropachymic acid (**29**),[30] polyporenic acid C (**30**),[31] 16α-hydroxy-24-methylene-3-oxolanost-8-en-21-oic acid (**31**),[31] 16α-acetoxy-24-methylene-3-oxolanost-8-en-21-oic acid (**32**),[31] 3-*epi*pachymic acid

(**33**),[32] palustrisoic acid H (**34**),[15] fomitoside K (**35**)[33] by comparing their NMR spectroscopic data with compounds reported in the literatures. The structure elucidation of thirteen new compounds was shown as below.

The molecular formula of **1** was determined to be $C_{36}H_{56}O_7$ on the basis of the HRESIMS ($[M - H]^-$, *m/z* 599.3976; calcd 599.3953). Its ^{1}H NMR spectrum showed eight singlet methyl protons at δ_H 0.78 (s), 0.88 (s), 0.92 (s), 0.95 (s), 1.02 (s), 1.36 (s), and 1.57 (s), one oxygenated methine proton at δ_H 4.66 (brs) and one olefinic proton at δ_H 5.09 (m). The ^{13}C-DEPT NMR spectra of **1** displayed thirty-six carbon resonances, attributing to eight methyls, twelve methylenes, five methines (one oxygenated and one olefinic) and eleven quaternary carbons (one ester carbonyl, two carboxyl, one oxygenated and three olefinic carbons). Notably, in the HMBC spectrum of **1**, two methylene protons (δ_H 2.70, m, H_2-2' and δ_H 2.65, m, H_2-5') and one methyl proton (δ_H 1.36, s, H_3-4') correlated with the oxygenated quaternary carbon (δ_C 70.7, C-3'). Together, the HMBC correlations of H-2' with the ester carbonyl (δ_C 172.4, C-1') and of H-5' with the carboxyl (δ_C 174.8, C-6') were observed, suggesting the presence of a 3-hydroxy-3-methylglutaroyl group. Additionally, the remaining thirty carbons were assigned to be a lanostan-8,24-dien-21-oic acid skeleton, which was proved by the HMBC, HSQC and ^{1}H-^{1}H COSY crossing peaks and was similar with fomitopsic acid.[11] Furthermore, the HMBC correlation of H-3 with C-1' indicated that the 3-hydroxy-3-methylglutaroyl group was connected to C-3.

The relative configuration of H-3 was established to be β based on the typical

shifts of C-3 and couple mode (Figure 2) of H-3 in the A-ring.[25,30,34] Research showed when H-3 was *β*-oriented, H-3 was a broad singlet and the chemical shift of C-3 was at δ_C 75–78 *ppm*; whereas, H-3*α* was in corresponding to a *dd* (*J* = 3–4 and 11–12 Hz) or *t* peak (*J* = 7–8 Hz) and C-3 was at δ_C 78–81 *ppm*. [25,30,34] On the basis of the mevalonate biosynthetic origin of moiety A and the comparison of the 1D NMR spectroscopic data with fomitopsic acid, C-3' was finally established to be *S*-configuration.[25,34] Thus, compound **1** was determined to be 3*α*-[(3'*S*)-4'-carboxyl-3'-hydroxy-3'-methylbutanoyloxy]-lanosta-8,24-dien-21-oic acid and named as formipinic acid A (**1**).

Compound **2** had a molecular formula of $C_{37}H_{58}O_8$ determined by HRESIMS at *m/z* 629.4073 $[M - H]^-$ (calcd 629.4059). Its 1D NMR spectroscopic data were similar to those of **1**, except for the presence of an additional methoxyl and an oxygenated methine in **2**. On the basis of the HMBC correlation of -OCH_3 with C-6', the additional methoxyl was located at C-6'. Similarly, combined typical shifts and HMBC correlations indicated that 4-methoxycarboxyl-3-hydroxy-3-methylbutanoyl was existed in **2**. In addition, the oxygenated methine proton (δ_H 4.60, m) showed the HMBC correlations with C-13 (δ_C 45.7), C-14 (δ_C 51.1), C-16 (δ_C 39.2), C-17 (δ_C 46.5), and C-30 (δ_C 17.6), which illustrated that the hydroxyl was linked to C-15. Meanwhile, 15*α*-OH was assigned on the basis of the ROESY correlation of H-15/H_3-18. A broad singlet signal at δ_H 4.92 of H-3 indicated that H-3 was *β*-oriented. Finally, the structure of **2** was determined to be 3*α*-[(3'*S*)-4'-methoxylcarbony-3'-hydroxy-3'-methylbutanoyloxy]-15-hydroxy-lanosta-

8,24-dien-21-oic acid and named as formipinic acid B (**2**).

The molecular formula of compound **3** was determined to be $C_{37}H_{60}O_7$ by the HRESIMS ([M + COOH]$^-$, *m/z* 661.4338; calcd 661.4321). Its 1D NMR spectra resembles those of **2**, with only difference in the replacement of the carboxyl at C-21 by an oxygenated methylene. Furthermore, the HMBC correlations of H_2-21 (δ_H 3.91, m; δ_H 4.07, d, J = 10.0 Hz) with C-17 (δ_C 43.6), C-20 (δ_C 43.9) and C-22 (δ_C 30.5), as well as the ^{1}H-^{1}H COSY correlations of H-21/H-20/H-23/H-24, and of H-21/H-20/H-17/H-16/H-15 confirmed that C-21 was the oxygenated methylene. Similarly, H-3 was a broad singlet peak in the ^{1}H NMR spectrum of **3**, suggesting that H-3 was *β*-oriented. Therefore, compound **3** was established to be 3*α*-[(3'*S*)-4'-methoxylcarbonyl-3'-hydroxy-3'-methylbutanoyloxy]-15*α*-hydroxy-lanosta-8,24-dien-21-ol and named as formipinol (**3**).

The molecular formula of **4** was assigned as $C_{41}H_{64}O_{10}$ on the basis of the HRESIMS (*m/z* 715.4452 [M – H]$^-$; calcd 715.4427). Detailed comparison of 1D NMR spectroscopic data between **4** and **3** showed that they had the same triterpenoid skeleton and a 4-carboxymethyl-3-hydroxy-3-methylbutanoyloxyl moiety. However, four additional carbon signals belonging to two ester carbonyls, one methylene and one methoxyl were observed in 1D NMR spectra of **4**. In the HMBC spectrum of **4**, the methylene protons (δ_H 3.33, s) correlated with two ester carbonyls (δ_C 166.4 and δ_C 166.6), meanwhile, the methoxyl protons (δ_H 3.68, s) showed correlation with C-3" (δ_C 166.6), which illustrated the presence of a 3"-methoxylcarbonylpropionyl. Moreover, the obvious downfield shift of the oxygen-bearing methylene at C-21 and

317 the HMBC correlation of H_2-21 (δ_H 4.21, d, J = 11.8 Hz) with C-1" (δ_C 166.4)
318 indicated the linkage of C-1" with C-21 through an oxygen atom. Thus, the structure
319 of **4** was unambiguously determined to be
320 3*α*-[(3'*S*)-4'-methoxylcarbony-3'-hydroxy-3'-methylbutanoyl]-21-(3"-methoxylcarbon
321 ylpropinonyl)-15*α*-hydroxy-lanosta-8,24-dien and named as formipiniate (**4**).

322 Compound **5** had the same molecular formula ($C_{32}H_{50}O_5$), and 1D NMR
323 spectroscopic data as those of 3*β*-*O*-acetyl-16*α*-hydroxytrametenolic acid (**27**),[28]
324 suggesting that they had the same planar structure, which was confirmed by the
325 analysis of the HMBC, HSQC and COSY spectra. However, carefully comparison of
326 their ^{1}H NMR spectroscopic data showed that the signal assignable to H-3 appeared
327 as a broad singlet at δ_H 4.73 in **5**, rather than the doublet at δ_H 4.50 (dd, J = 12.0 Hz)
328 in **27**.[28] Moreover, analysis of their ^{13}C NMR spectra exhibited the upfield shift of C-3
329 (δ_C 80.8→δ_C 76.9). Above information indicated **5** was a 3-epimer of
330 3*β*-acetoxyl-16*α*-hydroxylanosta-8,24-dien-21-oic acid. Thus, the structure of **5** was
331 established to be 3*α*-acetoxyl-16*α*-hydroxylanosta-8,24-dien-21-oic acid and named
332 as formipinic acid C (**5**).

333 Compound **6** had a molecular formula of $C_{32}H_{48}O_5$, which was determined by the
334 HRESIMS (*m/z* 513.3578 $[M + H]^+$; calcd 513.3575). Its 1D NMR spectra resembles
335 those of **5** with the only difference in the replacement of the oxygenaged methine at
336 C-16 by the carbonyl (δ_C 217.9). Furthermore, the HMBC correlations of H_2-15 (δ_H
337 1.98, m), H-17 (δ_H 2.93, m) and H-20 (δ_H 2.46, m) with the carbonyl (δ_C 217.9), and
338 of H-17 and H-20 with C-21 (δ_C 177.2) confirmed above deduction. 3*α*-OAc was

assigned on the basis of the typical broad singlet of H-3. Therefore, the structure of **6** was established to be 3α-acetoxy-16-oxo-lanosta-8,24-dien-21-oic acid and named as formipinic acid D (**6**).

The molecular formula of compound **7** was deduced from HRESIMS at *m/z* 483.3133 $[M-H]^-$ (calcd 483.3116). The ^1H and ^{13}C NMR spectroscopic data of **7** showed similarities with those of pinicolic acid B[21] with a 3,16-dioxolanosta-8,24-dien-21-oic acid skeleton. However, an oxygentated methine (δ_H 4.18, m; δ_C 65.6) was observed in 1D NMR spectra of **7** instead of the methylene in the later. Both H-5 (δ_H 2.10, m) and H-7 (δ_H 2.46, m; δ_H 2.54, m) showed the COSY correlations with the oxygen-bearing methine proton (δ_H 4.18, m). Moreover, the oxygenated methine proton (δ_H 4.18, m) correlated with C-4 (δ_C 47.4), C-5 (δ_C 55.1), C-10 (δ_C 39.8), C-7 (δ_C 38.2) and C-8 (δ_C 130.8) in the HMBC spectrum of **7**, which confirmed that the hydroxyl was connected to C-6. And the relative configuration of H-6 was assigned as β by the ROESY correlation of H-6/H_3-19. Finally, the structure of **7** was determined to be 6α-hydroxy-3,16-dioxoxlanosta-8,24-dien-21-oic acid and named as formipinic acid E (**7**).

The HRESIMS spectrum of **8** gave an $[M-H]^-$ ion peak at *m/z* 481.2972 (calcd 481.2958), consistent with the molecular formula $C_{30}H_{42}O_5$. The 1D NMR spectroscopic data of **8** were similar with those of **7**, except for the presence of two double bonds in **8**. On the basis of the typical chemical shifts of four olefinic carbons (δ_C 129.3, d; δ_C 143.7, s; δ_C 138.1, s; δ_C 119.1, d), two double bonds were $\Delta^{7,8}$ and

$\Delta^{9,11}$. The further confirmation was established from the HMBC correlations of H-7 (δ_H 5.81, s) with C-5 (δ_C 56.1), C-6 (δ_C 66.8), C-8 (δ_C 143.7), C-9 (δ_C 138.1) and C-14 (δ_C 43.9), of H-11 (δ_H 5.43, m) with C-8 (δ_C 143.7), C-9 (δ_C 138.1), C-12 (δ_C 35.5), and C-13 (δ_C 42.8), together with the ^{1}H-^{1}H COSY correlations of H-5/H_2-6/H-7, and of H-11/H_2-12. In the ROESY spectrum, the correlation of H-6/H_3-19 determined the relative configuration of 6-OH to be *α*. Thus, the structure of **8** was evaluated as 6*α*-hydroxy-3,16-dioxolanosta-7(8),9(11),24-trien-21-oic acid and named as formipinic acid F (**8**).

Compound **9** was isolated as white powder. Its molecular formula was assigned to be $C_{37}H_{58}O_9$ on the basis of the HRESIMS (*m/z* 669.3984 $[M + Na]^+$, calcd for $C_{37}H_{58}O_9$, 669.3973). The ^{13}C-DEPT NMR spectra of **9** displayed thirty-seven carbon resonances, belonging to six methyls, one methoxyl, twelve methylenes (one oxygenated and one olefinic), nine methines (one olefinic and five oxygenated) and nine quaternary carbons (four olefinic and two ester carbonyls). Among them, five oxygen-bearing methine signals at δ_H 6.40 (d, J = 8.2 Hz, H-1'), δ_C 95.6 (C-1'); δ_H 4.19 (t, J = 8.2 Hz, H-2'), δ_C 73.8 (C-2'); δ_H 4.28 (t, J = 8.8 Hz, H-3'), δ_C 78.8 (C-3'); δ_H 4.31 (t, J = 8.8 Hz, H-4'), δ_C 71.2 (C-4'); δ_H 4.07 (t, J = 8.9 Hz, H-5'), δ_C 79.1 (C-5'), as well as one oxygen-bearing methylene signals at δ_H 4.36 (dd, J = 11.7 and 4.2 Hz, H-6'), δ_H 4.45 (dd, J = 9.6 Hz, H-6'), δ_C 62.2 (C-6') indicated the presence of a *β*-D-glucosyl, which was further confirmed by analyzing the HPLC result of the hydrolyzate and by comparing with renitent time of *β*-D-glucose reported in the literature.

In addition, analysis of the ^{1}H-^{1}H COSY, HMBC, HSQC led to the establishment of a 3,4-*seco*-lanosta-4(28),8,24-trien-3,21-dioate skeleton. In the HMBC spectrum of **9**, H_2-28 (δ_H 4.78, s; δ_H 4.94, s) showed correlations with C-4 (δ_C 147.4), C-29 (δ_C 22.9), and C-5 (δ_C 46.9), meanwhile, H_2-1 (δ_H 1.73, m) and H_2-2 (δ_H 2.08, m; δ_H 2.44, m) correlated with C-3 (δ_C 174.2), which confirmed that A ring was a 3,4-*seco* ring system. Moreover, the methoxyl proton and the anomeric proton at δ_H 6.40 (d, J = 8.2 Hz) of the glucosyl showed the HMBC correlations with C-3 and C-21, respectively, suggesting that the O-CH_3 and the glucosyl were connected with C-3 and C-21. Consequently, the structure of **9** was determined to be 3,4-*seco*-lanosta-4(28),8,24-trien-3-oate-21-oic acid 21-*O*-*β*-D-glucoside and named formipinioside (**9**).

Compound **10** had a molecular formula of $C_{40}H_{62}O_9$ determined from the HRESIMS (*m/z* 685.4243, $[M-H]^-$, calcd 685.4321). Its 1D NMR spectra showed the diagnostic resonances of an acetoxyl (δ_H 2.40, s, δ_C 21.3; δ_C 170.8) and a 4-methoxycarbonyl-3-hydroxy-3-methylbutanoyl (δ_C 171.8, C-1'; δ_C 44.6, C-2'; δ_C 69.7, C-3'; δ_C 27.2, C-4'; δ_C 45.0, C-5'; δ_C 172.1, C-6'; δ_C 51.7, OCH_3). The remaining signals were assigned as seven methyls, ten methylenes including one sp^2 methylene (δ_H 4.62, s; δ_H 4.75, s; δ_C 106.7), six methines including two oxygenated methines (δ_H 4.92, brs, δ_C 78.0; δ_H 4.98, d, J = 7.8 Hz, δ_C 79.1), and eight quaternary carbons including three sp^2 (δ_C 133.5; δ_C 134.6; δ_C 154.8) and one carboxyl (δ_C 180.3) carbons. These data revealed that compound **10** was a lanostane-type C31 triterpenoid and was similar with palustrisoic acid H (**34**).[15] However, the hydroxyl at C-16 in the

later was replaced by an acetoxyl, which was confirmed by the HMBC correlations of H-16 (δ_H 4.98, t, J = 7.8 Hz) with C-14 (δ_C 48.0), C-15 (δ_C 40.1), C-17 (δ_C 52.8), C-13 (δ_C 45.0), and C-20 (δ_C 46.0), together with the ^{1}H-^{1}H COSY correlations of H-15/H-16/H-17/H-20/H-21. The broad singlet suggested an α-orientation of H-3. The ROESY correlations of H-16/H-18 indicated the α-orientation of 16-OAc. Thus, compound **10** was assigned as 16α-acetoxy-3α-[(3'*S*)-4'-methoxycarbonyl-3'-hydroxy-3'-methylbutanoyloxy]-24-met hyllanosta-8,24(31)-dien-21-oic acid and has been named forpinic acid A (**10**).

On the basis of the quasi-molecular ion peak at *m/z* 643.4235 $[M - H]^-$ (calcd 6434215) in the negative HRESIMS, the molecular formula of compound **11** was determined to be $C_{38}H_{60}O_8$. Detailed comparison of the 1D NMR spectroscopic data between **11** and palustrisoic acid H (**34**) showed that compound **11** was similar with palustrisoic acid H (**34**).[15] However, the obvious upfield shift of C-17 (δ_C 57.2→δ_C 46.5) and the oxygen-bearing methine (δ_C 77.5→δ_C 72.2), as well as the downfield shift of C-14 (δ_C 49.2→δ_C 51.9) were observed in the ^{13}C-DEPT NMR spectra of **11**, which indicated that the substituted positions of the hydroxyl in **11** and palustrisoic acid H could be different. Furthermore, the proton of the oxygenated methine proton (δ_H 4.62, m) showed a series of HMBC correlations with C-8 (δ_C 135.0), C-13 (δ_C 45.2), C-14 (δ_C 51.9), C-16 (δ_C 39.2), C-17 (δ_C 46.5), and C-30 (δ_C 19.0), illustrating that the hydroxyl was located at C-15. Moreover, 15α-OH was determined by the ROESY correlation of H-15/H_3-18. Therefore, the structure of **11** was established to be 15α-hydroxy-3α-[(3'*S*)-4'-methoxycarbonyl-3'-hydroxy-3'-

methylbutanoyloxy]-24-methyllanosta-8,24(31)-dien-21-oic acid and named as forpinic acid B (**11**).

Compound **12** gave an $[M + COOH]^-$ peak at *m/z* 805.4770 (calcd 805.4744) in its HRESIMS, showing that its molecular formula was $C_{43}H_{68}O_{11}$. The 1D NMR spectra of **12** exhibited that it was a C31 lanostane glycoside. Meanwhile, the ^1H NMR spectrum of **12** showed a doublet anomeric proton signal at δ_H 5.39(J = 7.7 Hz). Moreover, analysis of the COSY correlations suggested the presence of a *β*-D-xylose unit in **12**, which was identified by comparing the retention time of hydrolysis product with the standard substance using HPLC. The xylose moiety was attached at C-21 through an ester linkage by a key HMBC correlation of H-1" (δ_H 5.39, d, J = 7.7 Hz) with C-21 (δ_C 177.3). These data indicated that the structure of **12** was the same as that of fomitoside G.[12] Additionally, the replacement of the acetoxyl at C-3 in the later by a 4-methoxycarbonyl-3-hydroxy-3-methylbutanoyloxyl group was confirmed based on the presence of the characteristic NMR spectroscopic data and the HMBC correlation of H-3 (δ_H 4.66, brs) with C-1' (δ_C 172.3). Consequently, compound **12** was assigned as 3*α*-[(3'*S*)-4'-methoxycarbonyl-3'-hydroxy-3'-methylbutanoyloxy] -24-methyllanosta-8,24(31)-dien-21-oic acid 21-*O*-*β*-D-xylopyranoside and named as forpinioside A (**12**).

The molecular formula of compound **13** was $C_{31}H_{46}O_5$ determined from the HRESIMS (*m/z* 497.3258, $[M - H]^-$, calcd 497.3272). Its IR spectrum showed the presence of hydroxyl (3438 cm^{-1}), carbonyl (1709 cm^{-1}) and *α*,*β*-unsaturated carbonyl (1655 cm^{-1}) groups, which was consistent with the UV absorption band at 251 nm.

449 The ^{13}C-DEPT NMR spectra of **13** exhibited signals for ketone carbonyl (δ_C 213.6),
450 α,β-unsaturated carbonyl (δ_C 197.4), oxygen-bearing methine (δ_C 76.6) and terminal
451 double band (δ_C 155.8 and δ_C 106.8). These data indicated that **13** had the same
452 structure as 3β,16α-dihydroxy-7-oxo-24-methyllanosta-8,24(31)-dien-21-oic acid.[35]
453 The only difference was in the ketone instead of the oxygenated methine at C-3,
454 which was confirmed by the HMBC correlations of H_2-1 (δ_H 1.51; δ_H 1.77), H_2-2 (δ_H
455 2.36; δ_H 2.63), H_3-28 (δ_H 1.01), H_3-29 (δ_H 1.03), and H-5 (δ_H 2.13) with the ketone (δ_C
456 213.6). The relative configuration of 16-OH was assigned as α based on the ROESY
457 correlation of H-16/H_3-18. Thus, the structure of **13** was determined to be
458 16α-hydroxy-3,7-dioxo-24-methyllanosta-8,24(31)-dien-21-oic acid and named as
459 forpinic acid C (**13**).

460 All isolates were tested for their inhibitory activities against HL-60, A549,
461 SMMC-7721, MCF-7, and SW480 cell lines using MTS method and the results were
462 shown in Table 3. Compounds **12**, **14**, **17**, **18**, **22** and **23** showed cytotoxic effects
463 against five human tumor cell lines with IC_{50} values ranging from 3.92–28.51 μM.
464 Meanwhile, compounds **9** and **35** exhibited selected inhibitory activities against
465 HL-60, SMMC-7721 and MCF-7 with the IC_{50} values in the range of 13.57–36.01
466 μM. Among them, compound **14** displayed comparable cytotoxicity against HL-60
467 cell lines with IC_{50} value of 3.92 ± 0.2 μM, compared to the positive control (DDP,
468 IC_{50}: 1.09 ± 0.09 μM).

469 Since compounds **9**, **12**, **14**, **17**, **18**, **22**, **23** and **35** inhibited proliferation of
470 HL-60 cells, the ability of these compounds to induce cell death in HL-60 cells was

examined. The results showed that compounds **9**, **17**, **18** and **22** increased the percentage of apoptotic cells by 15.8%, 53.0%, 8.8% and 24.1%, respectively, at the concentration of 20 μM (Figure 4).

On the basis of cytotoxicity results, some preliminary structure-cytotoxicity relationships were proposed. The carboxyl of C-21 was a key for the cytotoxicity. Whatever the skeleton is C30 or C31, the presence of unsubstituted carboxyl at C-21 led to no active. However, when the carboxyl was replaced by the oxygenated methylene or glycosyl, the substituent of C-3 played a crucial role in cytotoxicity. Like compound **20**, it had the same structure as **22**, with the only difference in the replacement of *β*-OH by *α*-OAc in **20**. Compound **22** showed moderate cytotoxic activity against five tumor cell lines, nevertheless, compound **20** had no cytotoxicity. In addition, even though C-3 was substituted by OAc, OA or OB, compounds **3** and **4** having a 15-OH also were inactive.

In summary, thirty-five lanostane triterpenoids and triterpene sugar esters containing thirteen new compounds (**1**–**13**) and twenty-two known ones (**14**–**35**) were gained from the fruiting bodies of *F. pinicola*. Their structural types involved in C30 and C31 lanostane-type triterpenoids. Part of them showed moderate and weak cytotoxicities. Interestingly, all compounds possessing the unsubsituted carboxyl group at C-21 were inactive. However, previous study showed that pachymic acid and dehydroeburiconic acid had inhibitory effects against breast cancer (MDA-MB-231) and prostate cancer (DU145, LNCaP), and human colon carcinoma (HT-29),[36] which suggested that these isolates with a carboxyl group at C-21 may inhibit the

proliferation of above cancer cells. In the future, we will focus on the mechanism research of active component (compound **14**) and cytotoxicity analysis of lanostane-triterpenoid acids. These findings further indicate the significant potential of lanostane triterpenoids and their sugar esters in anti-tumor activity, and provide scientific evidence for the development and utilization of *F. pinicola* in food and drug industry.

ASSOCIATED CONTENT

Supporting Information

1D, 2D NMR and HRESIMS spectra of compounds **1**–**13** are included in supporting information.

AUTHOR INFORMATION

Corresponding Authors

*Telephone: +86-0871-5223327. Fax: +86-0871-5223325. E-mail: mhchiu@mail.kib.ac.cn

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Notes

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Table 1. ^{1}H and ^{13}C NMR Spectroscopic Data (600/150 MHz) of Compounds 1–7. (δ: *ppm*)

position	**1**[a]		**2**[b]		**3**[b]		**4**[c]		**5**[c]		**6**[c]		**7**[b]	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1	1.49 (m)	32.1	1.37 (m); 1.62 (m)	31.2	1.47 (m); 1.77 (m)	31.2	1.38 (m); 1.73 (m)	30.5	1.41 (m); 1.76 (m)	30.7	1.34 (m); 1.50 (m)	30.7	1.64 (m)	35.2
2	1.94 (m)	24.2	1.75 (m); 1.81 (m)	23.4	1.77 (m); 1.84 (m)	23.5	1.57 (m); 1.82 (m)	23.1	1.64 (m); 1.84 (m)	23.2	1.65 (m); 1.79 (m)	23.3	2.34 (m); 2.73 (m)	33.3
3	4.66 (brs)	79.8	4.92 (brs)	78.1	4.94 (brs)	78.0	4.66 (brs)	78.4	4.56 (brs)	77.8	4.83 (brs)	77.7		218.2
4		37.8		36.7		36.8		36.7		36.7		36.9		47.4
5	1.53 (m)	46.8	1.75 (m)	45.7	1.75 (m)	45.7	1.39 (m)	45.0	1.47 (m)	45.3	1.65 (m)	45.4	2.10 (m)	55.1
6	1.53 (m); 1.62 (m)	19.1	1.48 (m)	18.4	1.48 (m)	18.3	1.38 (m); 1.57 (m)	17.8	1.47 (m)	17.8	1.96 (m)	20.9	4.18 (m)	65.6
7	1.94 (m); 2.04 (m)	27.0	2.51 (m); 2.66 (m)	27.0	2.51 (m); 2.66 (m)	27.3	2.06 (m); 2.17 (m)	26.3	2.03 (m); 2.66 (m)	26.0	2.08 (m)	25.7	2.46 (m); 2.54 (m)	38.2
8		136.0		135.0		135.1		133.0		133.8		135.9		130.8
9		135.4		134.8		134.8		135.1		134.6		135.4		133.6
10		38.1		36.7		37.2		36.5		36.8		37.3		39.8
11	1.98 (m);	21.8	1.94 (m)	21.1	2.03 (m); 2.13 (m)	21.1	1.58 (m); 1.94 (m)	24.4	2.05 (m); 2.22 (m)	20.4	2.00 (m)	25.6	1.92 (m)	19.9

12	2.01 (m) 1.45 (m)	30.1	1.66 (m); 1.92 (m)	31.2	1.46 (m); 1.85 (m)	31.4	1.73 (m)	30.5	1.25 (m); 1.38 (m)	28.9	2.16 (m)	28.9	2.05 (m); 2.19 (m)	28.8
13		45.4		45.7		45.2		44.8		46.0		43.5		43.5
14		50.7		51.1		52.2		51.6		48.2		44.5		44.2
15	1.23 (m); 1.64 (m)	31.4	4.60 (m)	72.4	4.56 (m)	72.5	4.15 (m)	73.1	1.31 (m); 2.20 (m)	42.5	1.98 (m); 2.50 (m)	46.6	2.57 (d, J = 17.9 Hz); 2.07 (d, J = 17.9 Hz)	46.5
16	1.34 (m)	28.0	2.20 (m)	39.2	2.20 (m)	39.7	1.67 (m); 1.89 (m)	38.6	4.09 (m)	77.2		217.9		217.4
17	2.02 (m)	48.5	2.58 (s)	46.5	2.35 (m)	43.6	1.85 (m)	43.2	2.14 (m)	56.9	2.93 (m)	57.7	2.97 (m)	57.6
18	0.78 (s)	16.5	1.16 (s)	16.7	0.95 (s)	16.6	0.69 (s)	16.2	0.74 (s)	18.8	1.10 (s)	17.1	1.09 (s)	17.1
19	1.02 (s)	19.5	0.96 (s)	18.8	1.01 (s)	18.0	0.93 (s)	17.4	1.59 (s)	17.3	0.91 (s)	18.7	0.89 (s)	20.3
20	2.20 (m)	49.1	2.61 (m)	48.8	1.70 (m)	43.9	1.51 (m)	39.4	2.46 (m)	46.2	2.46 (m)	33.9	2.94 (m)	45.0
21		180.5		178.7	3.91 (m); 4.07 (d, J = 10.0 Hz)	61.8	3.97 (m); 4.21 (d, J = 11.8 Hz)	65.5		180.5		177.2		177.0
22	1.50 (m)	33.7	1.75 (m); 1.91 (m)	33.2	1.46 (m); 1.77 (m)	30.5	1.21 (m); 1.73 (m)	29.9	1.76 (m)	32.1	2.35 (m); 2.43 (m)	32.8	2.07 (m)	31.7
23	1.94 (m)	26.9	2.20 (m); 2.48 (m)	26.9	2.18 (m); 2.28 (m)	25.4	1.92 (m); 1.97 (m)	20.6	1.94 (m)	26.0	2.34 (m)	26.6	2.34 (m)	26.6
24	5.09 (m)	124.8	5.24 (m)	124.8	5.26 (m)	125.4	4.99 (m)	124.0	5.11 (m)	123.4	5.34 (m)	124.7	5.33 (m)	124.6
25		132.9		131.6		130.7		131.5		132.4		131.8		132.6
26	1.57 (s)	17.8	1.57 (s)	18.0	1.58 (s)	18.0	1.52 (s)	17.5	1.59 (s)	17.6	1.59 (s)	17.6	1.59 (s)	17.7
27	1.66 (s)	25.9	1.61 (s)	25.7	1.64 (s)	25.7	1.61 (s)	25.6	1.68 (s)	25.7	1.61 (s)	25.7	1.61 (s)	25.6

28	0.95 (s)	24.8	0.84 (s)	21.8	0.85 (s)	21.3	0.86 (s)	21.7	0.91 (s)	21.3	0.83 (s)	21.7	1.72 (s)	20.5
29	0.88 (s)	28.4	0.96 (s)	27.8	0.97 (s)	27.9	0.79 (s)	27.2	0.86 (s)	27.5	0.91 (s)	27.6	1.64 (s)	31.7
30	0.92 (s)	22.2	1.25 (s)	17.6	1.24 (s)	17.7	0.91 (s)	16.8	1.17 (s)	25.2	0.91 (s)	17.5	1.15 (s)	24.7
1'		172.4		171.1		171.1		171.6						
2'	2.70 (m)	45.5	2.96 (m)	46.5	2.96 (m)	46.2	2.60 (m)	44.7						
3'		70.7		69.7		69.7		69.5						
4'	1.36 (s)	27.9	1.63 (s)	28.3	1.64 (s)	28.3	1.29 (s)	27.5						
5'	2.65 (m)	45.7	3.02 (m)	45.8	3.02 (m)	45.8	2.67 (m)	44.7						
6'		174.8		171.7		171.8		171.9						
6'-OCH_3			3.59 (s)	51.1	3.58 (s)	51.1	3.63 (s)	51.5						
1"								166.4						
2"							3.33 (s)	41.3						
3"								166.6						
3"-OCH_3							3.68 (s)	52.3						
O-$\underline{C}OCH_3$										170.8		170.2		
O-$CO\underline{CH_3}$									2.07 (s)	21.8	1.94 (s)	20.3		

[a] Measured in CD_3OD; [b] Measured in C_5D_5N; [c] Measured in $CDCl_3$. The assignments were based on COSY, HSQC, and HMBC experiments.

Table 2. ^{1}H and ^{13}C NMR Spectroscopic Data (600/150 MHz) of Compounds 8–13. (δ: *ppm*)

position	**8**[b]		**9**[b]		**10**[c]		**11**[b]		**12**[a]		**13**[b]	
	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1	1.82 (m); 1.99 (m)	35.4	1.73 (m)	32.8	1.41 (m); 1.57 (m)	30.7	1.37 (m); 1.89 (m)	31.3	1.48 (m); 1.65 (m)	32.1	1.51 (m); 1.77 (m)	35.0
2	1.57 (m); 1.82 (m)	23.1	2.08 (m); 2.44 (m)	29.4	1.60 (m); 1.87 (m)	23.3	1.75 (m); 1.85 (m)	23.4	1.62 (m)	24.2	2.36 (m); 2.63 (m)	33.9
3		216.7		174.2	4.72 (brs)	78.5	4.92 (brs)	78.0	4.66 (brs)	79.8		213.6
4		47.3		147.4		36.8		37.1		37.7		47.0
5	1.98 (m)	56.1	2.14 (m)	46.9	1.44 (m)	45.3	1.75 (m)	45.7	1.52 (m)	46.7	2.13 (m)	50.2
6	4.61 (d, *J* = 10.1 Hz)	66.8	1.54 (m); 1.74 (m)	23.9	1.44 (m); 1.55 (m)	17.8	1.51 (m); 1.59 (m)	18.3	1.54 (m); 1.62 (m)	19.1	2.43 (m); 2.57 (m)	37.1
7	5.81 (s)	129.3	1.39 (m)	26.8	1.95 (m); 2.00 (m)	25.8	2.52 (m); 2.64 (m)	27.0	1.40 (m)	27.9		197.4
8		138.1		138.8		133.5		135.0		135.2		139.3
9		143.7		129.7		134.6		134.8		136.3		162.6
10		37.4		40.3		36.6		36.8		38.0		39.3
11	5.43 (m)	119.1	1.99 (m); 2.18 (m)	21.9	1.97 (m)	20.4	1.95 (m); 2.03 (m)	20.9	2.00 (m)	21.8	2.12 (m)	23.3
12	2.59 (d, *J* = 18.0 Hz); 2.71 (d, *J* = 18.0 Hz)	35.5	2.12 (m)	28.9	1.43 (m); 1.83 (m)	28.9	1.23 (m)	29.9	1.46 (m); 1.57 (m)	29.9	1.98 (m); 2.13 (m)	28.8
13		42.8		44.6		45.0		45.2		45.5		46.3
14		43.9		50.5		48.0		51.9		50.6		46.5

15	2.17 (d, *J* = 18.0 Hz); 2.56 (d, *J* = 18.0 Hz)	46.4	1.17 (m); 1.57 (m)	30.7	1.24 (d, *J* = 14.1 Hz); 2.26 (dd, *J* = 7.8, 14.1 Hz)	40.1	4.62 (m)	72.2	1.47 (m); 1.64 (m)	31.5	2.68 (m); 2.82 (m)	45.4
16		216.6	1.99 (m)	25.8	4.98 (t, *J* = 7.8 Hz)	79.1	2.21 (m)	39.2	2.06 (m)	27.0	4.50 (t, *J* = 7.0 Hz)	76.6
17	2.97 (m)	57.3	2.38 (m)	47.4	2.41 (m)	52.8	2.66 (m)	46.5	2.10 (m)	48.1	2.73 (m)	55.7
18	1.01 (s)	16.9	1.07 (s)	16.3	0.78 (s)	17.3	1.78 (s)	16.7	0.78 (s)	16.6	1.05 (s)	17.3
19	0.99 (s)	23.5	0.82 (s)	22.2	0.96 (s)	18.8	0.96 (s)	17.9	1.01 (s)	19.4	1.16 (s)	17.6
20	2.94(m)	44.8	2.68 (m)	48.0	2.48 (m)	46.0	2.62 (m)	48.9	2.36 (m)	48.6	2.89 (m)	48.4
21		177.0		175.7		180.3		178.6		177.3		179.6
22	2.53 (m); 2.61 (m)	33.9	1.84 (m)	33.3	1.58 (m); 1.69 (m)	30.0	1.89 (m); 2.03 (m)	31.7	1.64 (m)	32.6	2.44 (m); 2.64 (m)	31.3
23	2.34 (m)	26.6	1.93 (m)	26.2	1.99 (m)	31.5	2.27 (m); 2.39 (m)	32.6	1.92 (m); 2.06 (m)	32.6	2.37 (m); 2.50 (m)	33.1
24	5.32 (t, *J* = 6.4 Hz)	124.6	5.18 (m)	124.5		154.8		155.7		156.6		155.8
25		131.6		131.7	2.17 (m)	34.0	2.22 (m)	34.0	2.21 (m)	35.0	2.26 (m)	33.9
26	1.58 (s)	17.6	1.59 (s)	17.6	0.98 (d, *J* = 5.4 Hz)	21.8	0.96 (overlapped)	21.9	0.98 (overlapped)	22.1	0.97 (d, *J* = 6.7 Hz)	21.6
27	1.61 (s)	25.7	1.59 (s)	25.6	1.00 (d, *J* = 5.4 Hz)	21.8	0.96 (overlapped)	21.9	0.98 (overlapped)	22.1	0.97 (d, *J* = 6.7 Hz)	21.7
28	1.62 (s)	21.8	4.78 (s); 4.94 (s)	114.0	0.85 (s)	27.2	0.84 (s)	21.9	0.87 (s)	28.3	1.01 (s)	21.1
29	1.67 (s)	30.7	1.71 (s)	22.9	0.91 (s)	21.7	0.94 (s)	27.8	0.92 (s)	24.7	1.03 (s)	25.0
30	1.05 (s)	24.9	0.90 (s)	25.2	1.10 (s)	24.7	1.26 (s)	19.0	0.93 (s)	22.5	1.54 (s)	25.8
31					4.62 (s); 4.75 (s)	106.7	4.83 (s); 4.87 (s)	106.9	4.68 (s); 4.74 (s)	107.4	4.83 (s); 4.97 (s)	106.8
1'			6.40 (d, *J* = 8.2 Hz)	95.6		171.8		171.7		172.3		

2'	4.19 (t, *J* = 8.2 Hz)	73.8	2.71 (m)	44.6	2.96 (m)	45.8	2.69 (m)	45.9
3'	4.28 (t, *J* = 8.8 Hz)	78.8		69.7		69.7		70.8
4'	4.31 (t, *J* = 8.8 Hz)	71.2	1.36 (s)	27.2	1.63 (s)	28.6	1.35 (m)	28.1
5'	4.07 (d, *J* = 8.9 Hz)	79.1	2.65 (m)	45.0	3.01 (m)	46.2	2.69 (overlapped)	46.4
6'	4.36 (dd, *J* = 11.7, 4.2 Hz); 4.45 (dd, *J* = 9.6 Hz)	62.2		172.1		171.7		173.0
6'-OCH_3			3.65 (s)	51.7	3.58 (s)	51.1	3.62 (s)	51.9
1"							5.39 (d, *J* = 7.7 Hz)	96.4
2"							3.33 (m)	73.6
3"							3.36 (m)	78.0)
4"							3.50 (m)	70.9
5"							3.29 (m); 3.87 (dd, *J* = 11.5, 5.2 Hz)	67.6
O-$\underline{C}OCH_3$				170.8				
O-$CO\underline{CH_3}$			2.04 (s)	21.3				
3-OCH_3	3.63 (s)	51.2						

[a] Measured in CD_3OD; [b] Measured in C_5D_5N; [c] Measured in $CDCl_3$. The assignments were based on COSY, HSQC, and HMBC experiments.

Table 3. Cytotoxicity Data of Compounds 9, 12, 14, 17, 18, 22, 23, and 35 (IC_{50}: μM)

Compounds	HL-60	A549	SMMC-7721	MCF-7	SW480
9	15.48 ± 0.34	> 40	32.59 ± 0.78	26.05 ± 1.18	> 40
12	11.42 ± 0.39	19.95 ± 0.41	14.68 ± 0.26	21.06 ± 0.76	17.90 ± 1.17
14	3.92 ± 0.21	22.19 ± 0.76	12.81 ± 0.17	18.73 ± 0.53	16.35 ± 1.37
17	16.20 ± 0.21	17.52 ± 0.27	13.62 ± 0.27	15.72 ± 0.14	15.26 ± 0.29
18	14.67 ± 0.22	26.58 ± 1.10	28.51 ± 0.92	27.07 ± 2.43	27.05 ± 0.73
22	11.47 ± 0.49	16.74 ± 0.09	14.10 ± 0.19	12.40 ± 0.65	14.08 ± 0.55
23	11.10 ± 0.81	23.87 ± 0.71	18.03 ± 0.38	21.30 ± 0.48	21.77 ± 1.06
35	13.57 ± 0.85	> 40	36.01 ± 0.75	25.79 ± 1.69	> 40
DDP	1.09 ± 0.03	14.08 ± 2.89	2.68 ± 0.19	11.86 ± 0.93	9.60 ± 0.61
Paclitaxel	< 0.008	< 0.008	0.199 ± 0.077	< 0.008	< 0.008

DDP (*cis*-platinum) and Paclitaxel: positive control

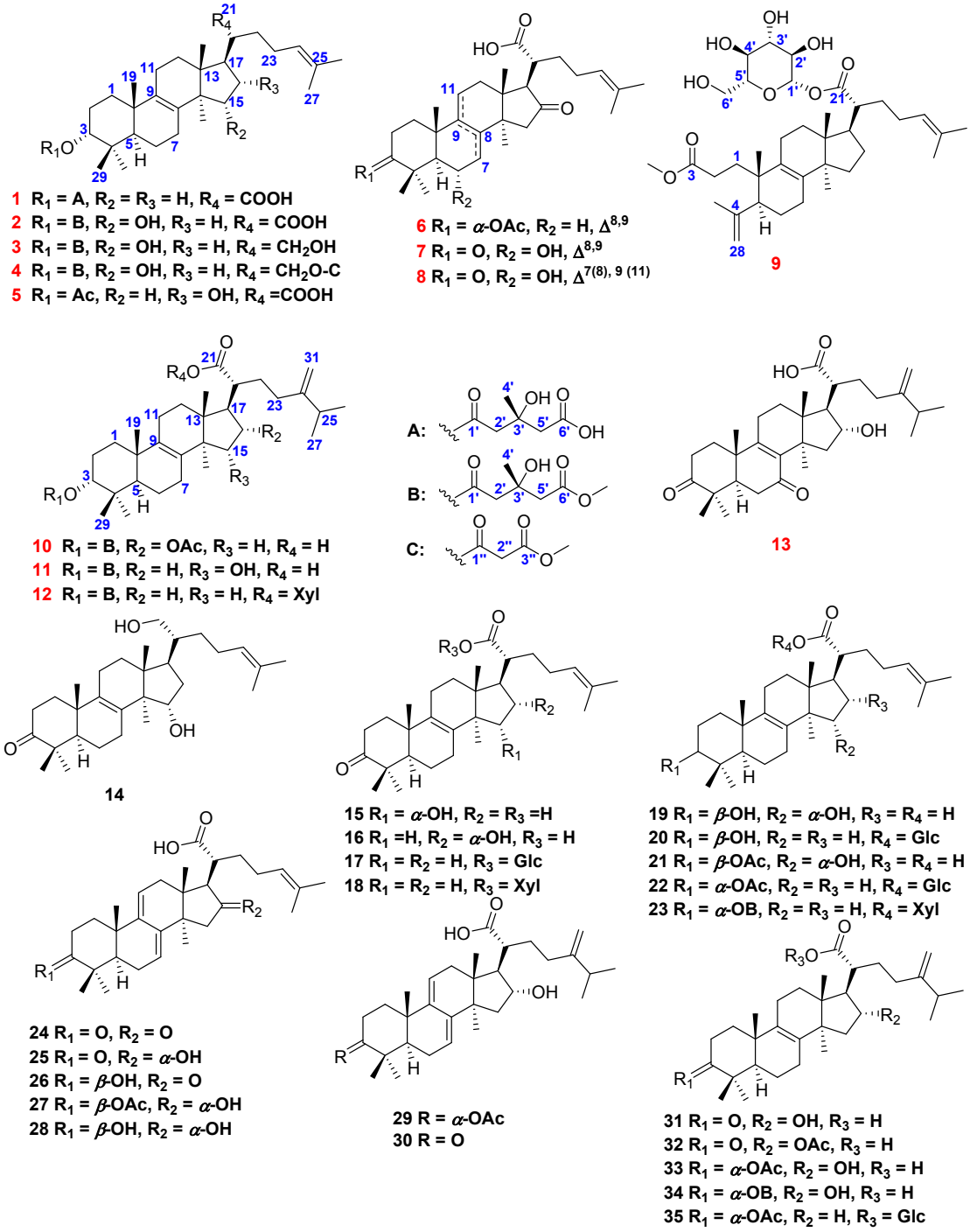

Figure 1. Structures of compounds **1–35** from the fruiting bodies of *Fomitopsis pinicola* (red: new compounds).

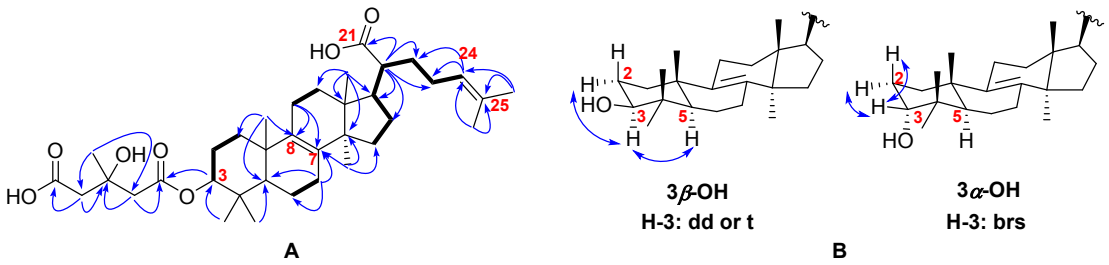

Figure 2. A: Selected HMBC (H→C) and ^{1}H-^{1}H COSY (H—H) correlations of compound **1**. B: Couple mode and ROESY correlations of H-3α and H-3β in lanostane-type triterpenoids.

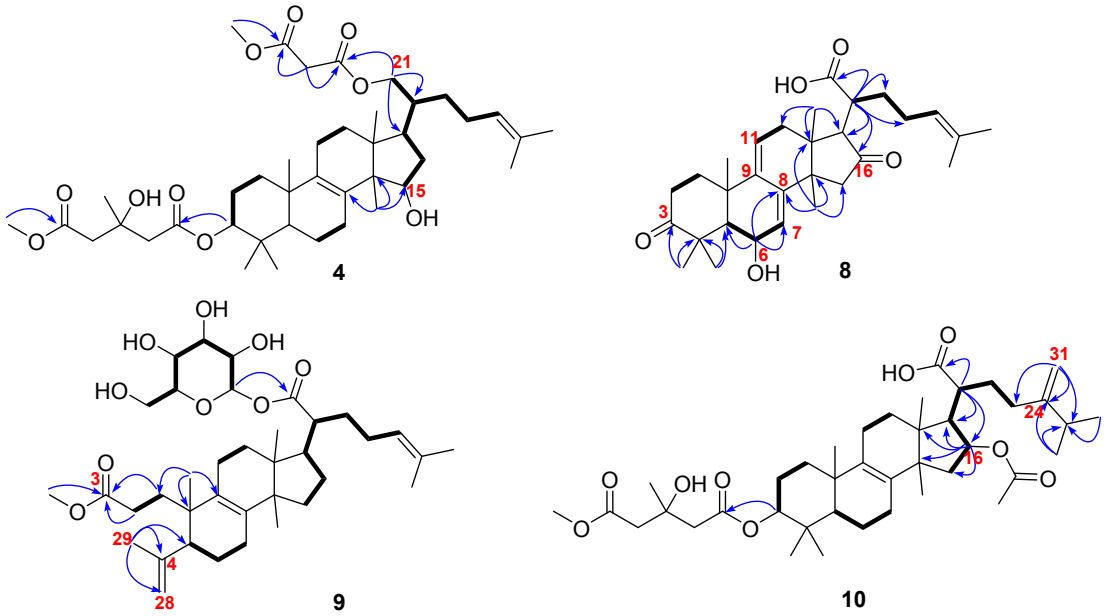

Figure 3. Key HMBC (H→C) and ^{1}H-^{1}H COSY (H—H) correlations of compounds **4**, **8**, **9** and **10.**

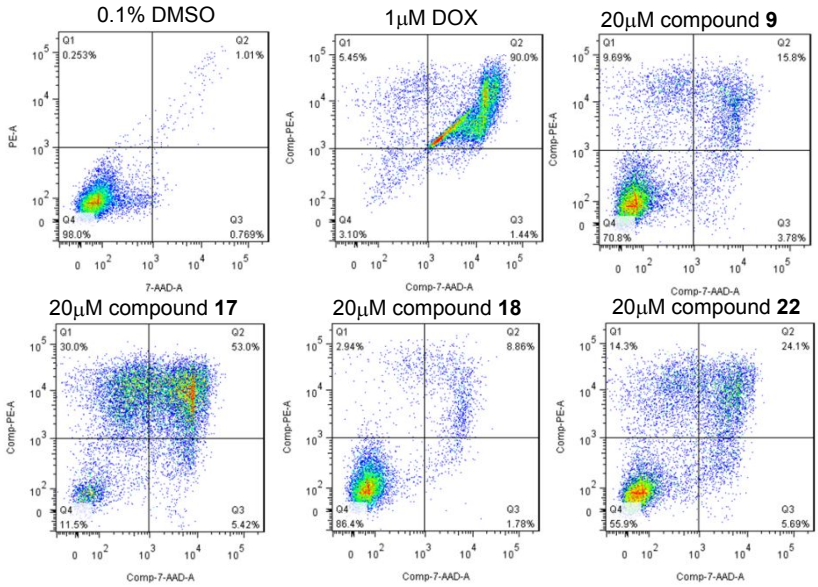

Figure 4. Effects of Doxorubicin and tested compounds **9**, **17**, **18** and **22** on apoptosis induction in HL-60 cells. Cells were treated with DMSO, doxorubicin and tested compounds for 24 h, the stained with annexin V-PE/7-AAD and analyzed with flow cytometry. (Q1: the early stage of apoptosis, Q2: the late stage of apoptosis, Q4: live cells).

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C30 and C31 Triterpenoids and Triterpene Sugar Esters with Cytotoxic Activities from Edible Mushroom *Fomitopsis pinicola* (Sw. Ex Fr.) Krast

Xing-Rong Peng,[†] Hai-Guo Su,[†,‡] Jun-Hong Liu,[†,‡] Yan-Jie Huang,[†,‡] Xin-Zhi Yang,[†,‡] Zhong-Rong Li [†], Lin Zhou [†] and Ming-Hua Qiu*[,†]

[†]State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Science.

[‡]University of the Chinese Academy of Science, Beijing