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SHORT COMMUNICATION

Cytotoxicity and Synergistic Effect of the Constituents from Roots of *Aglaia odorata* (Meliaceae)

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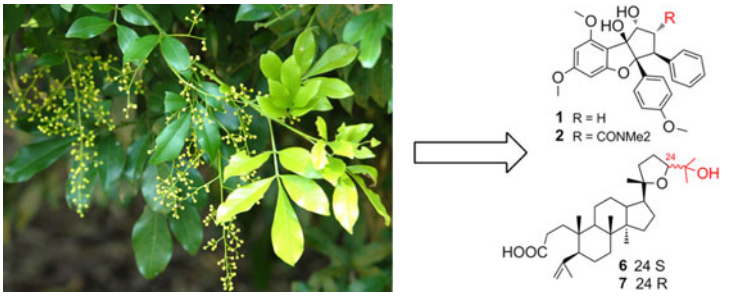

Twelve compounds were isolated from the roots of *Aglaia odorata.* Their structures were established on the basis of NMR and MS data as rocaglaol (**1**), rocaglamide (**2**), eichlerialactone (**3**), sapelins A (**4**), isofouquierone (**5**), eichlerianic acid (**6**), shoreic acid (**7**), agladupol E (**8**), 3-epimeliantriol (**9**), cleomiscosins B (**10**), 2β,3β-dihydroxy-5α-pregnane-16-one (**11**) and β-D-glucopyranos-1-yl *N*-methylpyrrole-2-carboxylate (**12**). Among them, compounds **1** and **2** showed significant cytotoxicity against human cancer cell (HL-60, SMMC-7721, A-549, MCF-7 and SW480) with IC_{50} values of 0.007–0.095 μM, while compounds **3**–**5** and **10** and **11** showed moderate to no cytotoxicity (IC_{50} 0.43 to values >40 μM). Compound **6** showed only weak cytotoxicity (IC_{50} 6.87 to >40 μM) and its epmier **7** was completely inactivite (IC_{50} > 40 μM) in the assay. However, potent synergistic effect was observed when the molar ratio of **6** to **7** is between 4:1 and 1:1.

Keywords: *Aglaia odorata*; cytotoxicity; flavaglines; epimer; synergistic effect

1. Introduction

The genus *Aglaia* (Meliaceae) comprised about 120 species primarily distributed in tropical and subtropical Asia as well as tropical Australia and Pacific islands, with eight species being distributed in China (Peng & Caroline 2008). During the past decades, species in the genus have attracted considerable attention as a prolific source of new natural products with promising bioactive. A number of flavaglines, triterpenoids and other compounds have been isolated from *Aglaia* (Ishibashi et al. 1993; Ohse et al. 1996; Nugroho et al. 1999; Cai et al. 2005; Su et al. 2006; Ebada et al. 2011; Zhang et al. 2012; Pan et al. 2014). *Aglaia odorata*, known as Mi-Zi-Lan or Shu-Lan in Chinese folklore, has been used as heart stimulant and febrifuge, or as an herb to treat cough, inflammations, injuries and toxin-causing vomiting (Proksch et al. 2001).

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In previous study, some flavaglines (Ohse et al. 1996), triterpenoids (Wang & Yang 2013; Liu et al. 2014), aminopyrrolidine-diamides (Hayashi et al. 1982; Inada et al. 2001) and essential oils (Joycharat et al. 2014) have been reported from *A. odorata*. In the current investigation, 12 compounds (**1**–**12**) including 2 epimeric (**6** and **7**) were isolated from the roots of *A. odorata*. The cytotoxicity of the 12 compounds against five human cancer cell lines and synergistic cytotoxicity effect of the epimers **6** and **7** were investigated.

2. Results and discussion

2.1. *Chemical identification*

Twelve compounds were isolated from the roots of *A. odorata*, and their structures were determined by comparison of their spectral data reported. They are rocaglaol (**1**) (Su et al. 2006), rocaglamide (**2**) (Ishibashi et al. 1993), eichlerialactone (**3**) (Phongmaykin et al. 2008), sapelins A (**4**) (Jolad et al. 1980), isofouquierone (**5**) (Waterman & Ampofo 1985), two epimers – eichlerianic acid (**6**) and shoreic acid (**7**) (Roux et al. 1998), agladupol E (**8**) (Xie et al. 2007), 3-epimeliantriol (**9**) (Lyons & Taylor 1975; Xie et al. 2007), cleomiscosins B (**10**) (Ray et al. 1982), 2β,3β-dihydroxy-5α-pregnane-16-one (**11**) (Inada et al. 1997) and β-D-glucopyranos-1-yl *N*-methylpyrrole-2-carboxylate (**12**) (Pailee et al. 2011) (see Figure 1).

2.2. *Cytotoxicity*

All compounds were evaluated for their cytotoxic activities against the five human tumour cell lines (A-549, MCF-7, SW480, SMMC-7721 and HL-60). The results (Table 1) indicated that rocaglaol (**1**) and rocaglamide (**2**) exhibited significant cytotoxic activities against the five human tumour cell lines, with IC_{50} values 0.007–0.095 μM. Compound **5** showed cytotoxic activities with IC_{50} values between 0.43 and 2.65 μM, while the other compounds **3**, **4**, **6**, **10** and **11** showed only weak or no cytotoxic activities, and compounds **7**–**9** and **12** showed no cytotoxicity ($IC_{50} > 40$ μM) in the assay (Tables 1 and 2).

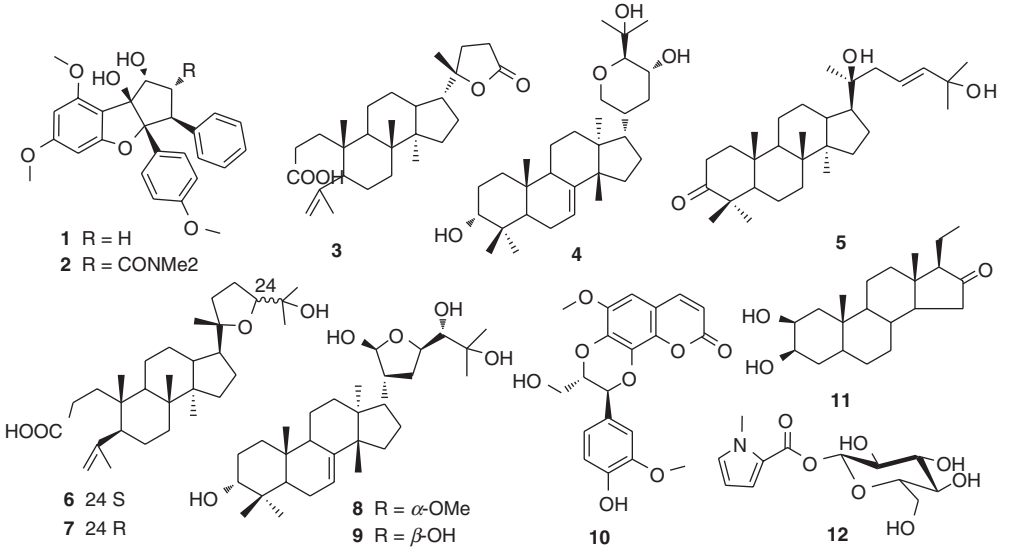

Figure 1. Structures of isolated compounds **1**–**12**.

Table 1. Cytotoxicity of isolated compounds from roots of *A. odorata* against five human cancer cell lines (IC_{50}, μM).

Substances	HL-60	SMMC-7721	A-549	MCF-7	SW480
1	0.039	0.093	0.095	0.008	0.040
2	0.007	0.009	0.008	0.008	0.031
3	8.97	9.50	5.95	2.03	35.92
4	29.57	NA	25.35	29.82	NA
5	1.60	1.16	0.98	0.43	2.65
6	16.15	13.69	7.80	6.87	NA
7	NA	NA	NA	NA	NA
10	3.56	4.16	3.46	1.69	13.30
11	9.49	10.65	6.08	9.77	NA
6a (**6**: **7** = 3:2)[a]	0.48	0.45	0.32	0.34	3.02
8, **9**, **12**	NA	NA	NA	NA	NA
Cisplatin[b]	2.03	13.54	12.56	18.65	19.70
Paclitaxel[b]	<0.008	<0.008	<0.008	<0.008	<0.008

Note: NA, not active ($IC_{50} > 40$ μM).
[a] **6a** was obtained as 3:2 mixture as measured by ^{1}H NMR peak integration, see S1.
[b] Positive control.

Table 2. Molar ratio-dependent cytotoxicity (IC_{50} μM) of the combination of epimers **6** and **7** against five human cancer cell lines.

Substances	Five human cancer cell lines				
6: **7**	HL-60	SMMC-7721	A-549	MCF-7	SW480
1:0	16.15	13.69	7.80	6.87	NA
4:1	0.95	1.04	1.06	2.69	0.76
7:3	0.38	0.67	0.43	0.79	0.53
1:1	0.64	0.71	0.69	1.62	0.45
3:7	NA	NA	NA	NA	NA
1:4	NA	NA	NA	NA	NA
0:1	NA	NA	NA	NA	NA
Cisplatin[a]	2.42	14.75	13.61	14.78	14.17

Note: NA, not active ($IC_{50} > 40$ μM).
[a] Positive control.

2.3. *Synergistic effect of the emiperics 6 and 7*

Although compound **6** showed only weak (IC_{50} 6.87–16.15 μM) or no cytotoxicity ($IC_{50} > 40$ μM) and its epimer **7** was completely inactive ($IC_{50} > 40$ μM, Table 2), pronounced cytotoxicity was observed for their mixtures containing molar ratio of 3:2 (**6** and **7**) (IC_{50} of 0.32–0.48 μM for cell lines except SW480, Table 1). Further investigation showed that the two epimers possessed potent synergistic effect against the five human cancer cell lines when the molar ratio of **6** to **7** was between 4:1 and 1:1 (Table 2). Dramatic cytotoxicity enhancement (IC_{50} 0.45–0.76 μM) was also observed for the SW480 cell line, which was in essence resistant to both epimers ($IC_{50} > 40$ μM, Table 1). The mechanisms governing the molar ratio-dependent cytotoxic effects are under investigation.

3. Conclusions

The cytotoxicity activity-guided chromatographic separation of *A. odorata* led to isolation of 12 compounds (**1**–**12**), which are as follows: two flavaglines (**1** and **2**), seven triterpenoids (**3**–**9**),

one coumarinolignan (**10**), one steroid (**11**) and one alkaloid (**12**). Among them, flavaglines (**1** and **2**) showed significant cytotoxicity against five human cancer cell lines (HL-60, SMMC-7721, A-549, MCF-7 and SW480) with values in the range of 0.007–0.039 μM, and some triterpenoids (**3**–**6**), coumarinolignan (**10**) and steroid (**11**) exhibited moderate to no cytotoxicity with values from 0.43 to values >40 μM. So it is suggested that flavaglines and triterpenoids are the major types of compounds responsible for the significant anticancer activities of *A. odorata*. The epimeric triterpenoids (**6** and **7**) exhibited potent synergistic cytotoxicity with values (IC_{50}) ranging from 0.38 to 2.69 μM with weight ratio (**6**:**7**) from 4:1 to 1:1. Compounds **4**, **5** and **8**–**12** are reported for the first time from the genus *Aglaia*.

Supplementary material

Supplementary material relating to this article is available online: experiments (S1), ^{1}H and ^{13}C NMR spectra of **6**, **7**, two mixtures **6a** (**6**:**7** = 3:2), **7a** (**6**:**7** = 2:3) S2 and S3.

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