Taylor & Francis
Taylor & Francis Group

Natural Product Research
Formerly Natural Product Letters

ISSN: 1478-6419 (Print) 1478-6427 (Online) Journal homepage: http://www.tandfonline.com/loi/gnpl20

A new cytotoxic polycyclic polyprenylated acylphloroglucinol from *Garcinia nujiangensis* screened by the LC-PDA and LC-MS

Zhongyan Tang, Lihua Lu, Xiaoyong Zhou, Jie Shen, Weiping Song, Yuedong Tang & Zhengxiang Xia

To cite this article: Zhongyan Tang, Lihua Lu, Xiaoyong Zhou, Jie Shen, Weiping Song, Yuedong Tang & Zhengxiang Xia (2019): A new cytotoxic polycyclic polyprenylated acylphloroglucinol from *Garcinia nujiangensis* screened by the LC-PDA and LC-MS, Natural Product Research, DOI: 10.1080/14786419.2018.1539983

To link to this article: https://doi.org/10.1080/14786419.2018.1539983

NATURAL PRODUCT RESEARCH
https://doi.org/10.1080/14786419.2018.1539983

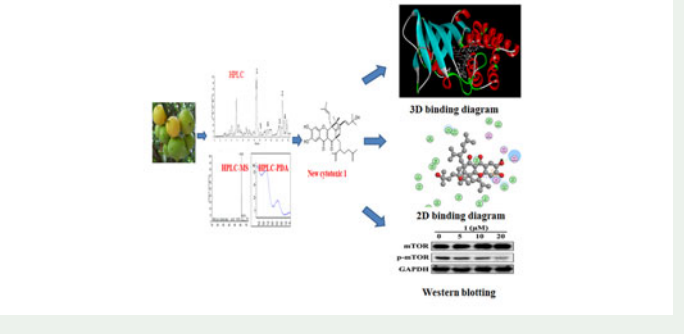
A new cytotoxic polycyclic polyprenylated acylphloroglucinol from *Garcinia nujiangensis* screened by the LC-PDA and LC-MS

Zhongyan Tang[a], Lihua Lu[b], Xiaoyong Zhou[a], Jie Shen[a], Weiping Song[a], Yuedong Tang[a] and Zhengxiang Xia[c]

[a]Department of Emergency and Critical Care Medicine, Jin Shan Hospital, Fudan University, Shanghai, China; [b]Department of Neonatology, Shanghai First Maternity and Infant Hospital, Tongji University School of Medicine, Shanghai, China; [c]Department of Pharmacy, Shanghai First Maternity and Infant Hospital, Tongji University School of Medicine, Shanghai, China

ABSTRACT

A new polycyclic polyprenylated acylphloroglucinol (**1**), nujiangefolin D, together with five known analogues (**2–6**), were isolated from the fruits of *Garcinia nujiangensis*. Compound **1** was screened by the LC-MS and LC-PDA. The structure of **1** was elucidated on the basis of extensive spectroscopic techniques including 1 D and 2 D NMR and MS analyses. The compounds isolated were evaluated for their cytotoxic activities against three cancer cell lines, **1** showed moderate cytotoxic activity against Hela, PANC-1, and MDA-MB-231 cell lines with IC_{50} values of 5.6 ± 0.1, 9.1 ± 0.2, and 8.3 ± 0.2 μM, respectively. The antitumor mechanism was explained via virtual docking of **1** to the main sites in the human serine/threonine-protein kinase mTOR (mTOR) crystal structure (PDB code: 4DRI). Furthermore, **1** may inhibit Hela cell proliferation through mTOR by the western blotting analysis. Taken together, **1** may be a potential mTOR inhibitor used for the treatment of cervical cancer.



ARTICLE HISTORY

Received 24 September 2018
Accepted 21 October 2018

KEYWORDS

Polycyclic polyprenylated acylphloroglucinol; cytotoxic; molecular docking; mTOR; *Garcinia nujiangensis*

CONTACT Jie Shen J1999sh@163.com; Zhengxiang Xia xzx5380537@163.com

Supplemental data for this article can be accessed at https://doi.org/10.1080/14786419.2018.1539983.

1. Introduction

Cancer is not only a major public health problem but also a leading cause of death in the world. A standard treatment for cancer is surgical resection or irradiation with adjuvant chemotherapy. In addition to rapid metastasis, problems such as a low response rate, a lack of selectivity towards cancer cells, and multidrug resistance have limited the success of chemotherapy. Therefore, there is a high demand for new anti-tumor agents that have high potency and selective toxicity towards cancer cells.

Polycyclic polyprenylated acylphloroglucinols (PPAPs) are a class of hybrid natural products sharing the mevalonate/methylerythritol phosphate and polyketide biosynthetic pathways and showing considerable structural and bioactive diversity (Richard et al. 2012; Wang et al. 2018; Yang et al. 2018; Guo et al. 2017). PPAPs isolated from *Garcinia* species are recognized for their structural diversity and significant biological activity (Ciochina and Grossman 2006), they will be used for the treatment of cancer, HIV, and depressant in future (Wu et al. 2014).

mTOR is a serine/threonine protein kinase in the PI3K-related kinase (PIKK) family that forms the catalytic subunit of two known as mTOR Complex 1 (mTORC1) and 2 (mTORC2) (Saxton and Sabatini 2017). These two complexes regulate fundamental cell physiology processes in response to distinct cellular inputs including growth factors and nutrients. While mTORC2 mainly controls cell proliferation and survival, mTORC1 governs protein and lipid synthesis, cell growth, proliferation, metabolism, and autophagy. In cancer mechanism study, mTORC1 functions as a downstream effector for many frequently mutated oncogenic pathways, including the PI3K/Akt pathway as well as the Ras/Raf/Mek/Erk (MAPK) pathway, resulting in mTORC1 hyperactivation in a high percentage of human cancers, which has led to the development of small-molecule inhibitors that target various nodes in the pathway, the first mTOR inhibitor approved for use in cancer was a class of rapamycin derivatives known as 'rapalogs'. However, these inhibitors have also shown some promise in preclinical and early clinical trial data but have raised concerns over dose-limiting toxicities (Rodrik-Outmezguine et al., 2016). Therefore, there is a critical need for novel therapeutic strategies for the management of cancer patients.

Conventional bio-guided isolated from medicinal plants often requires a large amounts of material and is both laborious and costly (Ji et al. 2017). Due to the structural similarity among the PPAPs derivatives, it is challenging to discover new analogues from *Garcinia* species (Li et al. 2016). Previously, we reported several anti-tumor PPAPs from the leaves (Xia et al. 2012) and twigs (Tang et al. 2015) of *G. nujiangensis*. Among them, two unusual new PPAPs named nujiangefolins A and B characterized by the enol hydroxyl formed a six-membered ring with a benzene ring carbon were isolated from the leaves of *G. nujiangensis*, their UV absorption peaks were approximately at λ_{max} 266 and 322 nm. In our ongoing search for new anti-tumor compounds from plants in *Garcinia*, a new strategy combined application of the LC-MS and LC-PDA was used to screen new PPAPs from the extract of the fruits of *G. nujiangensis*. Thus, a new PPAP and five known analogues were obtained, their activities were evaluated, and the possible anti-tumor molecular mechanism of **1** was explored. Herein, the screening, isolation, structure elucidation, cytotoxic activities, molecular docking, and molecular mechanism of these compounds were reported in this paper.

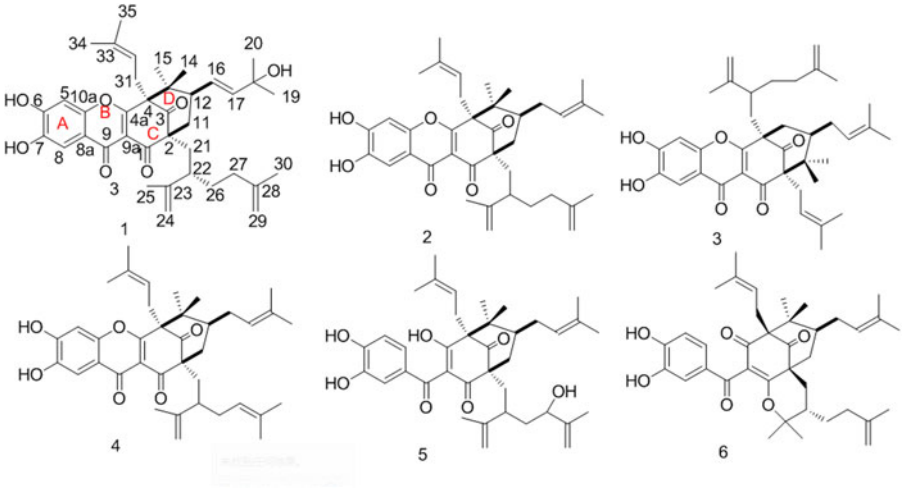


Figure 1. The structure of compounds from *Garcinia nujiangensis*.

2. Results and discussion

An acetone extract of the fruits of *G. nujiangensis* was defatted with petroleum ether, and the defatted residue was then separated by column chromatography over silica gel to yield nine fractions monitored by TLC. All fractions were analyzed by the LC-MS and LC-PDA using two modes: (1) an MS full scan, to obtain the molecular weight of detected peaks; and (2) a PDA full scan, to obtain the UV absorption peaks. An interest compound had the molecular weight 616 and the UV absorption peaks at 266 and 322 nm was discovered in this screening. It might have the molecular formula $C_{38}H_{48}O_7$, which was not searched in the SciFinder database (Chemical Abstracts Service, Columbus, OH, USA). As a result, it could be a new compound. To confirm this deduction, the fraction was separated and purified by column chromatography and HPLC to give compounds **1**–**6** (as displayed in Figure 1), and their structures were elucidated by spectroscopic methods including 1 D and 2 D NMR and MS analyses.

Compound **1** was isolated as a yellow gum. The molecular formula $C_{38}H_{48}O_7$ was deduced by HRESIMS at *m/z* 615.3316 $[M–H]^-$. Two singlet aromatic protons (δ_H 7.41 and 6.91) in the ^{1}H NMR spetrum indicated the presence of a 1,2,4,5-tetrasubstituted benzene ring (as shown in A ring). The ^{1}H NMR data suggested that **1** possessed seven olefinic protons and eight methyl groups. The analysis of the aromatic region of the ^{13}C NMR data revealed three oxygenated carbons at δ_C 147.2 (C-7), 155.2 (C-7), and 151.7 (C-10a). These data further confirmed the presence of a 1,2,4,5-tetrasubstituted aromatic A ring. Resonances gave six-membered ring, consisting of a non-conjugated ketone (δ_C 209.1) flanked by two quaternary carbons (δ_C 65.6 and 63.2) and an enolized 1,3-diketone (δ_C 120.5, 176.7, and 195.3), were observed in the ^{13}C NMR spectrum. The NMR data of **1** were similar to those of nujiangefolin A (Xia et al 2012), indicated that these two compounds had similar carbon skeletons. However, the different signals due to two olefinic carbons (δ_C 142.0 and 128.3) and one oxygenated carbon (δ_C 71.1) were observed in the ^{13}C NMR data of **1**. After carefully analysis of the HMBC, HSQC, and ^{1}H-^{1}H COSY spectra (as shown in Figure S1), the planar structure of

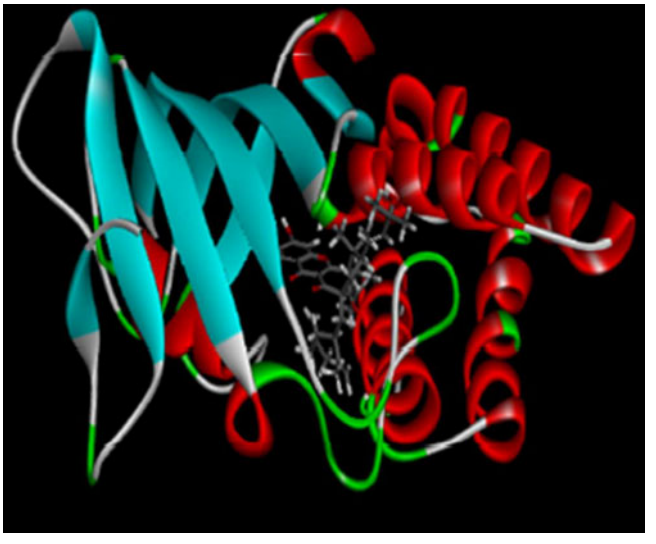


Figure 2. 3D binding diagram of 1.

1 (nujiangefolin D) was deduced as shown in Figure 1. The relative configurations were assigned based on the analysis of the ^{13}C NMR and NOESY spectra. The NOESY (Figure S1) correlations of H-31/H-15, H-15/H-12, H-12/H-21, and H-22/H-26 indicated that they were α oriented, and H-14/H-16 showed that they were in the opposite direction. The absolute configuration of **1** was determined as 2S, 4 R, 12S, 22 R by comparison of the experimental and calculated electronic circular dichroism (ECD) (as shown in Figure S12). Therefore, the structure of nujiangefolin D (**1**) was determined as displayed in Figure 1.

In addition to the new PPAP (**1**), five known analogues, such as, nujiangefolin A (**2**) (Xia et al. 2012), nujiangefolin B (**3**) (Xia et al. 2012), symphonone H (**4**) (Marti et al. 2010), garcimultiflorone E6 (**5**) (Ito et al. 1997), and (-)-cycloxanthochymol (**6**) (Wu et al. 2005) were also isolated and identified from *Garcinia nujiangensis* (as shown in Figure 1). The structures of the known compounds were identified by comparison of spectroscopic data with reported values.

The cytotoxicities of six PPAPs were evaluated against three human cancer cell lines including Hela, PANC-1, and MDA-MB-231. As summarized in Table S2, compound **1** exhibited moderate cytotoxic activities against Hela, PANC-1, and MDA-MB-231 cell lines with IC_{50} values of 5.6 ± 0.1, 9.1 ± 0.2, and 8.3 ± 0.2 μM, respectively. Compound **4** also showed moderate cytotoxic activities against Hela, PANC-1, and MDA-MB-231 cell lines with IC_{50} values of 10.3 ± 0.2, 8.4 ± 0.1, and 12.3 ± 0.1 μM, respectively. Other compounds showed selectivity toward these cancer cells. The antitumor mechanism was explained via virtual docking of **1** into the main sites in the human Serine/threonine-protein kinase mTOR (mTOR) crystal structure (pdb 4DRI). The docking study (as exhibited in Figure 2) showed that **1** could bind to the protein mTOR with lower binding energy than the original ligand rapamycin. The interplay between **1** and mTOR was shown in Figure S12, the hydroxyl of **1** could form a hydrogen bond with the mTOR ASP2102 residue, and the flat aromatic core of **1** could form π-π interactions and salt bridges with the mTOR. Moreover, **1** could inhibit Hela cell proliferation via mTOR by

the western blotting analysis (as shown in Figure S13). The results confirmed that **1** may be valuable to cancer therapy, which still need further investigation.

3. Experimental

3.1. General experimental procedures

HPLC analysis was run on an Agilent 1200 instrument equipped with multiple wavelength diode array detector (DAD) and Mass spectrometry (MS) equipped with an ESI source. Optical rotations were recorded with a JASCO P-1020 polarimeter. 1 D and 2 D NMR spectra using CD_3OD as solvents were taken on an Agilent 500MR-NMR spectrometer at 500 MHz (^1H) and 125 MHz (^{13}C). ECD spectra were recorded on a JASCOJ-810 spectrometer. Mass spectrometry was performed on a Waters Q-TOF Premier instrument (Micromass MS Technologies, Manchester, UK) equipped with an ESI source in the negative-ion mode. Column chromatography was performed with silica gel (200-300 mesh, Qingdao Haiyang Chemical Co., Ltd.), Sephadex LH-20 (Pharmacia), and reversed-phase C_{18} silica gel (250 mesh, Merck). Precoated TLC sheets of silica gel 60 GF_{254} (Qingdao Haiyang Chemical Plant, Qingdao, P.R. China) were used. An Agilent 1200 Series machine equipped with a Zorbax SB-C_{18} column (4.6 × 250 mm, 5 μm) was used for HPLC analysis, and a semi-preparative Zorbax SB-C_{18} column (9.4 × 250 mm, 5 μm) was used in sample preparation. Protein concentrations were measured using a Nano Drop 2000 spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). Cell viability was assayed by reading the absorbance of each well at 450 nm using a multifunction microplate reader (Scientific Vario, Thermo Scientific).

3.2. Plant material

The fruits of *G. nujiangensis* were collected in Nujiang, Yunnan Province, People's Republic of China, in August 2015. The plant material was identified by Prof. Yuanchuan Zhou, T. C. M. of Yunnan University. A voucher sample (G. N. 0006) was deposited in the translational Medicine Laboratory, Shanghai First Maternity and Infant Hospital, Tongji University School of medicine. Dulbecco's modified Eagle'smedium (DMEM) and 0.05% trypsin-ethylenediaminetetraacetic acid (EDTA) were obtained from Invitrogen (Grand Island, NY). Fetal bovine serum (FBS) was purchased from HyClone (Logan, UT). mTOR, p-mTOR (S2448), and GAPDH were purchased from Abcam (Cambridge, UK).

3.3. Extraction and isolation

The fruits of *G. nujiangensis* (600 g) were pulverized and extracted with acetone three times at room temperature. The acetone-soluble extract (60 g) was suspended in hot water and partitioned with CH_2Cl_2. The CH_2Cl_2-soluble extract (26 g) was then passed through a silica gel column (200–300 mesh, 200 g), eluted with CH_2Cl_2–MeOH in a gradient (1:0 to 0:1), to afford eight fractions A–H that were pooled according to their performance of TLC. Every fraction was screened by the LC-MS and LC-PDA, Fr.B (427 mg) was proposed that contained a new compound was further separated by

ODS with methanol-water (75:25 to 95:5) as eluant to give three subfractions B1–B3. Fr.B1 (20 mg) was purified by semi-preparative HPLC with methanol–water (75:25 containing 0.1% trifluoroacetic acid, 3 mL/min) to yield compounds **1** (10 mg, $t_R = 14.1$ min), **5** (2 mg, $t_R = 12.3$ min), and **6** (1 mg, $t_R = 9.8$ min). Fr.B2 (80 mg) was separated by semi-preparative HPLC with methyl methanol–water (85:15, containing 0.1% trifluoroacetic acid, 3 mL/min) to yield compounds **2** (5 mg, $t_R = 7.2$ min), **3** (2 mg, $t_R = 10.1$ min), and **4** (6 mg, $t_R = 8.1$ min).

Nujiangefolin D (**1**), yellow gum; [α]20 D −12 (*c* 0.10, MeOH); UV ($CHCl_3$) λ_{max} (log ε) 266 (4.27), 322 (4.01) nm; IR (thin film) $\nu_{max}cm^{-1}$: 3423, 3072, 2958, 2923, 1731, 1687, 1617, 1485, 1385, 1292, 1143, 894; ^1H and ^{13}C NMR, see Table S1; ESIMS(−): *m/z* 615($[M-H]^-$); HR-ESIMS(−): *m/z* 615.3316 (calcd for $C_{38}H_{47}O_7$ 615.3322).

3.4. Cell culture

Pancreatic cancer (PANC-1), cervical adenocarcinoma (HeLa), and breast cancer (MDA-MB-231) cells, obtained from American Type Culture Collection (Manassas, VA), were grown in antibiotic free DMEM supplemented with 10% FBS. The cells were maintained in a humid incubator (37 °C, 5% CO_2).

3.5. Cytotoxicity assay

The cytotoxicity bioassay of the compounds against human cancer cells (Hela, PANC-1, and MDA-MB-231) was determined *in vitro* by the MTT assay (Tang et al. 2018). Etoposide was used as the positive control drug.

3.6. Molecular docking

Crystal structures of human mTOR (PDB code: 4DRI) was obtained from the Protein Data Bank (http://www.rcsb.org). The virtual screening was implemented in the LibDock module of the Discovery studio software 3.0. Molecules were built with Chemdraw and optimized at molecular mechanical and semiempirical level by using Open Babel GUI. The crystallographic ligands were extracted from the active site and the designed ligands were modelled. All the hydrogen atoms were added to define the correct ionization and tautomeric states, and the carboxylate, phosphonate, and sulphonate groups were considered in their charged form. In the docking calculation, the default FlexX scoring function was used for exhaustive searching, solid body optimizing and interaction scoring. Finally, the ligands with the lowest-energy and the most favorable orientation were selected.

3.7. Western blot analysis

Hela cells were harvested and homogenized in 1 × RIPA lysis buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.25% deoxycholic acid, 1% NP-40, 1 mM EDTA, and phosphatase and protease inhibitors) for 30 min at 4 °C. Supernatants were collected, assayed for protein concentration by using a bicinchoninic acid protein (BCA) assay kit

(Beyotime, Haimen, China), aliquoted and stored at −80 °C until used for Western blotting. Equal amounts of total cellular proteins (40 *ug*) were separated by sodium dodecyl sulfate (10% or 12%) polyacrylamide gel electrophoresis (SDS-PAGE, BioRad Laboratories, Hercules, CA), transferred onto PVDF membranes (BioRad Laboratories,Hercules, CA) and then probed with primary antibody specifc for mTOR, p-mTOR, and GAPDH, followed by secondary antibody conjugated with horseradish peroxidase (Cell Signaling Technology). The immunocomplexes were visualized using ChemiDOC^{TM} XRS + system (BioRad Laboratories, Hercules, CA).

4. Conclusion

In this study, a new strategy combined application of the LC-MS and LC-PDA was used to screen the new antitumor compounds from the fruits of *G. nujiangensis*. Finally, a new cytotoxic polycyclic polyprenylated acylphloroglucinol named nujiangefolin D (**1**) together with five known analogues (**2**–**6**) were isolated, and their structures were elucidated by spectroscopic methods including 1 D and 2 D NMR and MS analyses. Moreover, the activity screening indicated that **1** showed moderate cytotoxic activity against Hela, PANC-1, and MDA-MB-231 cell lines with IC_{50} values of 5.6, 9.1, and 8.3 μM, respectively. The antitumor mechanism was explained via virtual docking of **1** to the main sites in the human Serine/threonine-protein kinase mTOR (mTOR) crystal structure (PDB code: 4DRI). Furthermore, **1** could inhibit Hela cell proliferation via mTOR by the western blotting analysis. In summary, **1** may be a potential mTOR inhibitor used for the treatment of cervical cancer. For clinical application, further research is needed to investigate the detailed molecular mechanisms underlying anticancer effects.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This research was supported in part by the Special subject for scientific research of Chinese Medicine from Shanghai Municipal Commission of Health and Family Planning (No. 2016JQ002 and 2018JP007) and Shanghai municipal medical and health discipline construction projects (No. 2017ZZ02015).

References

Ciochina R, Grossman RB. 2006. Polycyclic polyprenylated acylphloroglucinols. Chem Rev. 106(9): 3963–3986.

Guo Y, Zhang N, Chen CM, Huang JF, Li XN, Liu JJ, Zhu HC, Tong QY, Zhang JW, Luo ZW, et al. 2017. Tricyclic polyprenylated acylphloroglucinols from St John's Wort, Hypericum perforatum. J Nat Prod. 80(5):1493–1504.

Ito C, Miyamoto Y, Nakayama M, Kawai Y, Rao KS, Furukawa H. 1997. A novel depsidone and some new xanthones from *Garcinia* species. Chem Pharm Bull. 45(9):1403–1413.

Ji BK, Gao XM, Cui D, Wang SS, Huang WZ, Li YK, Mei SX, Yang Z, Li GP, Jiang M, et al. 2017. Two new biphenyls from the stems of *Garcinia tetralata*. Nat Prod Res. 31(13):1544–1550.

Li P, Senthilkumar HA, Figueroa M, Wu SB, Fata JE, Kennelly EJ, Long CL. 2016. UPLC-QTOFMS(E)-guided dereplication of the endangered Chinese species *Garcinia paucinervis* to identify additional benzophenone derivatives. J Nat Prod. 79(6):1619–1627.

Rodrik-Outmezguine VS, Okaniwa M, Yao Z, Novotny CJ, Whirter CM, Banaji A, Won H, Wong W, Berger M, Stanchina E, et al. 2016. Overcoming mTOR resistance mutations with a new-generation mTOR inhibitor. Nature. 534(7606):272–276.

Saxton RA, Sabatini DM. 2017. mTOR signaling in growth, metabolism, and disease. Cell. 168(6): 960–976.

Marti G, Eparvier V, Moretti C, Prado S, Grellier P, Hue N, Thoison O, Delpech B, Guéritte F, Litaudon M. 2010. Antiplasmodial benzophenone derivatives from the root barks of Symphonia globulifera (Clusiaceae). Phytochemistry. 71(8–9):964–974.

Richard JA, Pouwer RH, Chen DYK. 2012. The chemistry of the polycyclic polyprenylated acylphloroglucinols. Angew Chem Int Ed. 51(19):4536–4561.

Tang ZY, Xia ZX, Qiao SP, Jiang C, Shen GR, Cai MX, Tang XY. 2015. Four new cytotoxic xanthones from *Garcinia nujiangensis*. Fitoterapia. 102:109–114.

Tang ZY, Shen JM, Zhang F, Liang JY, Xia ZX. 2018. Sulfated neo-clerodane diterpenoids and triterpenoid saponins from Sheareria nana S. Moore. Fitoterapia. 124:12–16.

Wang WY, Liao YY, Huang XM, Tang C, Cai P. 2018. A novel xanthone dimer derivative with antibacterial activity isolated from the bark of *Garcinia mangostana*. Nat Prod Res. 32(15): 1769–1774.

Wu CC, Weng JR, Won SJ, Lin CN. 2005. Constituents of the pericarp of *Garcinia subelliptica*. J Nat Prod. 68(7):1125–1127.

Wu SB, Long CL, Kennelly EJ. 2014. Structural diversity and bioactivities of natural benzophenones. Nat Prod Rep. 31(9):1158–1174.

Xia ZX, Zhang DD, Liang S, Lao YZ, Zhang H, Tan HS, Chen SL, Wang XH, Xu HX. 2012. Bioassay-guided isolation of prenylated xanthones and polycyclic acylphloroglucinols from the leaves of *Garcinia nujiangensis*. J Nat Prod. 75(8):1459–1464.

Yang XW, Grossman RB, Xu G. 2018. Research progress of polycyclic polyprenylated acylphloroglucinols. Chem Rev. 118(7):3508–3558.