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Prenylated flavonoids from the fruits of *Sinopodophyllum emodi* and their cytotoxic activities†

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Thirteen new prenylated flavonoids, sinoflavonoids C–O (**1**–**13**), were isolated from the fruits of *Sinopodophyllum emodi* together with eleven known analogues (**14**−**24**). Their structures were elucidated on the basis of spectroscopic evidence. The cytotoxic activities of all isolated compounds were evaluated against MCF-7 and HepG2 cell lines. By the preliminary structure−activity relationships, it was firstly discovered that the simple, non-prenylated 5,7,3′,4′-tetrahydroxyflavonol analogues (**22** and **23**) showed higher cytotoxic activities than corresponding prenylated ones (**1**–**2**, **4**–**21**, and **3** and **24**). Compound **22** exhibited the most potent cytotoxicity against MCF-7 and HepG2 cell lines, with IC_{50} values of 3.14 and 2.08 μM, respectively.

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Introduction

Cancer is of international public health problem.[1] Although a large number of cancer chemotherapeutic agents are introduced into clinical use, their typical adverse effects are inevitably ubiquitous, such as anaemia, hair loss and severe gastrointestinal disturbances. In addition, drug resistance is also a major hindrance to effective treatment.[2] Chinese medicinal herbs, have became a promising source of potential new drugs. The dried ripe fruit of *Sinopodophyllum emodi* (Wall.) Ying (Berberidaceae) called "Xiaoyelian" in Chinese, is a well-known traditional Tibetan medicine for the treatment of amenorrhea, dead fetus, and placental retaining.[3] The plant is particularly rich in aryltetralin lactone lignans, and attracts wide attention due to their cytotoxic and antiviral properties.[3–9] As part of a program to search for cytotoxic natural products, we previously reported the isolation, characterization and cytotoxic activity of aryltetralin lactone and tetrahydrofuranoid lignans, and preparative isolation of prenylated flavonoids, sinoflavonoids A–B from *S. emodi*.[9–11] In a further examination of the fruits of this plant, thirteen new prenylated flavonoids, sinoflavonoids C–O (**1**–**13**), were obtained together with eleven known analogues (**14**−**24**). Details of the isolation, structure elucidation and cytotoxicity of all isolated flavonoids were reported herein (Fig.1).

Results and discussion

Compounds **1** and **2** were obtained as yellow, amorphous powders. Their HR-ESI-MS showed the same molecular formula of $C_{26}H_{28}O_7$, according to an $[M + Na]^+$ quasi-molecular ion peak [*m/z* 475.1731 in **1**; *m/z* 475.1737 in **2**]. The ^1H and ^{13}C NMR spectra of **1** and **2** (Table 1) were similar to each other in showing the presence of a 1,2,3,4-tetra-substituted [δ_H 6.85 (1H, d, J = 8.5 Hz), 6.73 (1H, d, J = 8.5 Hz) in **1**; δ_H 6.85 (1H, d, J = 8.2 Hz), 6.73 (1H, d, J = 8.2 Hz) in **2**] and penta-substituted benzene ring [δ_H 6.30 (1H, s) in **1**; δ_H 6.42 (1H, s) in **2**], two

oxygen-bearing olefinic carbons [δ_C 158.8, 139.1 in **1**; δ_C 158.06, 138.8 in **2**], a carbonyl group [δ_C 178.6 in **1**; δ_C 178.0 in **2**], a 3-methyl-2-butenyl [δ_H 5.04 (1H, t, J = 6.9 Hz), 3.23 (2H, d, J = 6.9 Hz), 1.48 (3H, s), 1.54 (3H, s), δ_C 21.4, 122.6, 131.3, 17.8, 25.8 in **1**; δ_H 5.15 (1H, t, J = 7.1 Hz), 3.22 (2H, d, J = 7.1 Hz), 1.71 (3H, s), 1.61 (3H, s), δ_C 20.9, 122.1, 130.7, 17.7, 25.5 in **2**], a 2,2-dimethyldihydropyrano group [δ_H 2.59 (2H, t, J = 6.7 Hz), 1.72 (2H, t, J = 6.7 Hz), 1.30 (6H, s), δ_C 20.5, 32.2, 74.3, 26.8 (×2) in **1**; δ_H 2.60 (2H, t, J = 6.7 Hz), 1.71 (2H, t, J = 6.7 Hz), 1.30 (6H, s), δ_C 20.1, 31.8, 73.9, 26.4 (×2) in **2**], a aromatic methoxy group [δ_H 3.57 (3H, s), δ_C 60.6 in **1**; δ_H 3.56 (3H, s), δ_C 60.2 in **2**], and three phenolic hydroxyl groups [δ_H 12.58 (1H, s), 10.79 (1H, s), 9.18 (1H, s) in **1**; δ_H 12.89 (1H, s), 10.87 (1H, s), and 9.20 (1H, s) in **2**]. These spectroscopic data indicated that **1** and **2** were prenylated flavanonol derivatives. The 3-methyl-2-butenyls in **1** and **2** were connected respectively to C-8 and C-6 by the HMBC correlations (Fig.2) of H-1″ (δ_H 3.23) with C-7 (δ_C 161.9), C-8 (δ_C 106.3), and C-9 (δ_C 154.5), and H-1″ (δ_H 3.22) with C-5 (δ_C 158.13), C-6 (δ_C 110.7), and C-7 (δ_C 161.9). The remaining HMBC correlations of **2** were similar to those of **1**. The 2,2-dimethyldihydropyrano groups were fused to C-2′ and C-3′ by the long range correlations from H-1‴ (δ_H 2.59) to C-1′ (δ_C 120.8), C-2′ (δ_C 121.2), and C-3′ (δ_C 142.3) in **1**, and H-1‴ (δ_H 2.60) to C-1′ (δ_C 120.3), C-2′ (δ_C 121.1), and C-3′ (δ_C 142.0) in **2**. The methoxy group at C-3 was proved, based on the HMBC correlation between methoxy group protons [δ_H 3.57 in **1**; δ_H 3.56 in **2**] and C-3 (δ_C 139.1 in **1**; δ_C 138.8 in **2**). Thus, compounds **1** and **2** were deduced respectively as

8-(3-methyl-2-butenyl)-2′,3′-(2,2-dimethyldihydropyrano)-5,7,4′-trihydroxy-3-metho xyflavone and 6-(3-methyl-2-butenyl)-2′,3′-(2,2-dimethyldihydropyrano)-5,7,4′-trihydroxy-3-metho xyflavone, and named sinoflavonoids C and D.

Compounds **3** and **4** were obtained as yellow, amorphous powders. Their ^{1}H and ^{13}C NMR (Tables 1 and 2) were quite similar to those of **1**, except that another phenolic hydroxyl group [δ_H 8.90 (1H, s) in **3**] and aromatic proton [δ_H 6.35 (1H, s) in **4**] were observed respectively instead of methoxy group and 3-methyl-2-butenyl in **1**. Those were further supported by their HR-ESI-MS, which gave an $[M + H]^+$ quasi-molecular ion peak [*m/z* 439.1760 in **3**; *m/z* 385.1262 in **4]**, being 14 and 68 mass-units less than that of **1**, respectively. Thus, compounds **3** and **4** were identified as 8-(3-methyl-2-butenyl)-2′,3′-(2,2-dimethyldihydropyrano)-3,5,7,4′-tetrahydroxyflavo ne and 2′,3′-(2,2-dimethyldihydropyrano)-5,7,4′-trihydroxy-3-methoxyflavone, and named sinoflavonoids E and F.

Compounds **5**–**7** were obtained as yellow, amorphous powders. Their molecular formulae were assigned as $C_{26}H_{28}O_7$ by HR-ESI-MS (*m/z* 453.1910 $[M + H]^+$ in **5**; *m/z* 491.1477 $[M + K]^+$ in **6**; *m/z* 453.1892 $[M + H]^+$ in **7**). Their ^{1}H, ^{13}C NMR (Tables 1 and 2) and HSQC spectra were similar to those of **1** and **2**. Two tertiary-methyl signals [δ_H 1.30 (6H, s), δ_C 26.3(×2) in **5**; δ_H 1.29 (6H, s), δ_C 26.3 (×2) in **6,** δ_H 1.31 (6H, s), δ_C 26.41 (×2) in **7**], two methylene groups [δ_H 2.61 (2H, t, J = 6.6 Hz), 1.81 (2H, t, J = 6.6 Hz), δ_C 15.7, 30.9 in **5**; δ_H 2.64 (2H, t, J = 6.9 Hz), 1.73 (2H, t, J = 6.9 Hz), δ_C 15.5, 31.8 in **6**; δ_H 2.54 (2H, t, J = 6.9 Hz), 1.74 (2H, t, J = 6.9 Hz), δ_C 16.7, 30.8 in **7**], and one oxygen-bearing aliphatic quaternary carbon [δ_C 76.2 in **5**; δ_C 76.3 in **6**; δ_C 74.8 in **7**] were observed, implying the presence of another

2,2-dimethyldihydropyrano group in **6**, and **5** and **7** instead of 3-methyl-2-butenyl in **1** and **2**, respectively. The 2,2-dimethyldihydropyrano groups in **5** and **7** were attached respectively to C-6 and C-7, and C-5 and C-6 by the HMBC correlations (Fig. 2) from H-1″ [δ_H 2.61 in **5**; δ_H 2.54 in **7**] to C-5 [δ_C 158.5 in **5**; δ_C 157.0 in **7**], C-6 [δ_C 104.3 in **5**; δ_C 105.0 in **7**], and C-7 [δ_C 159.8 in **5**; δ_C 159.8 in **7**], in combination with the presence of the phenolic hydroxyl group [δ_H 12.98 (1H, s, 5-OH) in **5**; δ_H 10.59 (1H, s, 7-OH) in **7**]. In contrast, the 2,2-dimethyldihydropyrano group in **6** was located at C-7 and C-8 by the HMBC correlations of H-1″ (δ_H 2.64) with C-7 (δ_C 159.5), C-8 (δ_C 100.0) and C-9 (δ_C 153.9). Based on those examinations, compounds **5**, **6** and **7** were elucidated respectively as 6,7-bis-2′,3′-(2,2-dimethyldihydropyrano)-5,4′-dihydroxy-3-methoxyflavone (**5**), 7,8-bis-2′,3′-(2,2-dimethyldihydropyrano)-5,4′-dihydroxy-3-methoxyflavone (**6**), 5,6-bis-2′,3′-(2,2-dimethyldihydropyrano)-7,4′-dihydroxy-3-methoxyflavone (**7**), and named sinoflavonoids G, H and I.

Compounds **8** and **9** were obtained as yellow, amorphous powders and possessed the same molecular formula $C_{26}H_{30}O_8$, as revealed from their HR-ESI-MS analyses (*m/z* 471.2024 $[M + H]^+$ in **8**; *m/z* 509.1581 $[M + K]^+$ in **9**). The ^1H, ^{13}C-NMR (Tables 1 and 2) and HSQC spectra of **8** were similar with those of **9**, showing the presence of a 1,2,3,4-tetra-substituted [δ_H 6.76 (1H, d, J = 8.2 Hz), 6.74 (1H, d, J = 8.2 Hz) in **8**; δ_H 6.74 (1H, d, J = 8.5 Hz), 6.72 (1H, d, J = 8.5 Hz) in **9**] and penta-substituted benzene ring [in each case, δ_H 6.29 (1H, s)], two oxygen-bearing olefinic carbons [δ_C 158.7, 138.5 in **8**; δ_C 158.8, 138.7 in **9**], a carbonyl group [δ_C

178.3 in **8**; δ_C 178.3 in **9**], a 3-methyl-2-butenyl [δ_H 4.98 (1H, t, J = 6.8 Hz), 3.30 (2H, d, J = 6.8 Hz), 1.27 (3H, s), 1.42 (3H, s), δ_C 25.6, 122.9, 130.2, 17.3, 25.3 in **8**; δ_H 5.02 (1H, t, J = 7.2 Hz), 3.21 (2H, d, J = 7.2 Hz), 1.44 (3H, s), 1.53 (3H, s), δ_C 21.2, 121.9, 130.8, 17.4, 25.4 in **9**], a 3-hydroxy-3-methylbutyl [δ_H 2.56 (2H, m), 1.45 (2H, m), 1.00 (6H, s), δ_C 17.3, 42.9, 68.8, 28.9 (×2) in **8**; δ_H 2.54 (2H, m), 1.41 (2H, m), 0.89 (6H, s), δ_C 22.3, 43.5, 68.6, 28.7 (×2) in **9**], a methoxy group [δ_H 3.53 (3H, s), δ_C 59.8 in **8**; δ_H 3.59 (3H, s), δ_C 60.0 in **9**], and four phenolic hydroxyl groups [δ_H 12.58 (1H, s), 10.68 (1H, s), 9.85 (1H, s), 8.51 (1H, s) in **8**; δ_H 12.58 (1H, s), 10.75 (1H, s), 9.77 (1H, s), 8.43 (1H, s) in **9**]. The 3-methyl-2-butenyls in **8** and **9** were assigned respectively to C-2′ and C-8 from the HMBC correlations of H-1‴(δ_H 3.30) with C-1′ (δ_C 121.3), C-2′ (δ_C 127.8), and C-3′ (δ_C 143.2), and H-1″ (δ_H 3.21) with C-7 (δ_C 161.4), C-8 (δ_C 105.8), and C-9 (δ_C 154.2). The HMBC spectra also showed respectively the correlations of H-1″ (δ_H 2.56) with C-7 (δ_C 161.6), C-8 (δ_C 107.2), and C-9 (δ_C 154.2), and H-1‴ (δ_H 2.54) with C-1′ (δ_C 121.4), C-2′ (δ_C 129.3), and C-3′ (δ_C 143.2), indicating that the 3-hydroxy-3-methylbutyls were attached to C-8 in **8** and C-2′ in **9**. The methoxy group was located at C-3, based on the HMBC correlations between methoxy group protons [δ_H 3.53 in **8**; δ_H 3.59 in **9]** and C-3 [δ_C 138.5 in **8**; δ_C 138.7 in **9**). Thus, compounds **8** and **9** were deduced respectively as 8-(3-hydroxy-3-methylbutyl)-2′-(3-methyl-2-butenyl)-5,7,3′,4′-tetrahydroxy-3-metho xyflavone and 8-(3-methyl-2-butenyl)-2′-(3-hydroxy-3-methylbutyl)-5,7,3′,4′-tetrahydroxy-3-metho xyflavone, and named sinoflavonoids J and K.

Compounds **10** and **11** were obtained as yellow, amorphous powders and possessed the same molecular formula $C_{26}H_{30}O_8$, as revealed from their HR-ESI-MS analyses (*m/z* 493.1836 $[M + Na]^+$ in **10**; *m/z* 471.2021 $[M + H]^+$ in **11**). Their ^1H and ^{13}C NMR spectra (Tables 1 and 2) were similar to those of **8** and **9**, except for the appearance of 2,2-dimethyldihydropyrano groups in **10** and **11** instead of 3-methyl-2-butenyls found in **8** and **9.** 2,2-Dimethyldihydropyrano groups were proved by two tertiary-methyl signals [δ_H 1.30 (6H, s), δ_C 26.5 (×2) in **10**; δ_H 1.29 (6H, s), δ_C 26.2 (×2) in **11**], two methylene groups [δ_H 2.68 (2H, t, J = 6.6 Hz), 1.73 (2H, t, J = 6.6 Hz), δ 20.3, 31.8 in **10**; δ_H 2.65 (2H, t, J = 6.4 Hz), 1.77 (2H, t, J = 6.4 Hz), δ_C 15.8, 30.9 in **11**], and an oxygen-bearing aliphatic quaternary carbon [δ_C 73.9 in **10**; δ_C 76.2 in **11**]. By the HMBC correlations of H-1‴ (δ_H 2.68) and H-1″ (δ_H 2.65) with C-1′ (δ_C 120.3), C-2′ (δ_C 120.9) and C-3′ (δ_C 141.9), and C-7 (δ_C 159.8), C-8 (δ_C 99.8) and C-9 (δ_C 153.9), 2,2-dimethyldihydropyrano groups in **10** and **11** were linked to C-2′ and C-3′, and C-7 and C-8, respectively. Thus, compounds **10** and **11** were deduced respectively as 8-(3-hydroxy-3-methylbutyl)-2′,3′-(2,2-dimethyldihydropyrano)-5,7,4′-trihydroxy-3-methoxyflavone and 7,8-(2,2-dimethyldihydropyrano)-2′-(3-hydroxy-3-methylbutyl)-5,3′,4′-trihydroxy-3-methoxyflavone, and named sinoflavonoids L and M.

The ^1H and ^{13}C NMR spectra of compounds **12** and **13** (Tables 1 and 2) were quite similar to those of **10** and **11**, respectively, except for the observation of a methoxy group [δ_H 2.94 (3H, s), δ_C 48.3 in **12**; δ_H 2.85 (3H, s), δ_C 48.2 in **13**], suggesting **12**

and **13** to be a further methyl ether derivative of **10** and**11**, respectively. This was further confirmed by the HR-ESI-MS of **12** and **13**, which gave the same molecular formula by a quasi-molecularion peak [*m/z* 507.1993 $[M + Na]^+$ in **12**; *m/z* 485.2157 $[M + H]^+$ in **13**], being 14 mass-units more than that of **10** and **11**. The additional methoxy groups were located at C-3″ in **12**, and C-3‴ in **13**, based on the HMBC correlations between methoxy group protons [δ_H 2.94 in **12**; δ_H 2.85 in **13**] and C-3″ (δ_C 73.6 in **12**; δ_C 73.5 in **13**). Thus, compounds **12** and **13** were determined respectively as 8-(3-methoxy-3-methylbutyl)-2′,3′-(dimethyldihydropyrano)-5,7,4′-tetrahydroxy-3-methoxyflavone and 7,8-(2,2-dimethyldihydropyrano)-2′-(3-methoxy-3-methylbutyl)-5,3′,4′-tetrahydroxy-3-methoxyflavone, and named sinoflavonoids N and O.

The isolated known flavonoids were identified as podoverine A (**14**),[12] 8-prenylquercetin-3-methyl ether (**15**),[13] 6-prenylquercetin-3-methyl ether (**16**),[14] 7,8-(2,2-dimethylpyrano)-2′-(3-methyl-2-butenyl)-5,3′,4′-trihydroxyl-3-methoxyflavone (**17**),[13] sinoflavonoid A (**18**),[11] sinoflavonoid B (**19**),[11] 8,2′-diprenylquercetin-3-methyl ether (**20**),[3] 8-(3-methyl-2-butenyl)-2′,3′-(2,2-dimethylpyrano)-5,7,4′-trihydroxyl-3-methoxyflavone (**21**),[13] quercetin-3-methyl ether (**22**),[15] quercetin (**23**),[3] and 8-prenylquercetin (**24**),[16] by comparison of their spectroscopic data with values reported in the literature.

All isolated compounds were evaluated for their in vitro cytotoxic activities against MCF-7 and HepG2 cell lines using the MTT assay with etoposide as a positive

control, and IC_{50} values were summarized in Table 3. Among them, only compounds **14** and **22** were cytotoxic, with IC_{50} values of less than 10μM. Compound **22** showed the highest cytotoxicities against MCF-7 and HepG2 cell lines, with IC_{50} values of 2.46 and 2.08 μM, respectively.

Conclusions

With diverse structure and extensive pharmacological activity, natural prenylated flavonoids have become the focus of natural products search. These compounds have a relatively narrow distribution in the plant kingdom, mostly from Leguminosae and Moraceae.[17] Nineteen flavonoids, including twelve prenylated ones, have been isolated from *S. emodi*.[3,11,13,18–19] A few of them were tested for the anti-proliferative activity in tumor cell lines.[3,13,19] The phytochemical studies on *S. emodi* resulted in the isolation of thirteen new prenylated flavonoids and eleven known analogues. Their cytotoxic activity was evaluated against MCF-7 and HepG2 cell lines. Based on these preliminary structure-activity results obtained by us, it was firstly discovered that the simple, non-prenylated 5,7,3′,4′-tetrahydroxyflavonol analogues (**22** and **23**) showed higher cytotoxic activities than corresponding prenylated ones (**1**–**2**, **4**–**21**, and **3** and **24**) with such side-chains (3-methyl-2-butenyl, 2,2-dimethyldihydropyrano group, 2,2-dimethylpyrano group, 3-methoxy-3-methylbutyl, 3-hydroxy-3-methylbutyl, and 2-hydroxy-3-methyl-3-butenyl). Compound **22** were the most interesting of all isolated compounds. Further studies are necessary to explore antitumor mechanism and cytotoxicities in normal cells.

Experimental

General experimental procedures

The UV spectra were measured on a Shimadzu UV-1700 spectrometer. The IR spectra were taken on a Bruker Tensor 27 Fourier transform infrared (FTIR) spectrometer with KBr discs. The 1D and 2D NMR spectra were recorded on Bruker-AC (E)-500 spectrometer with TMS as an internal standard. HR-ESI-MS was determined on a Bruker microTOF-Q instrument. The chromatographic silica gel (200–300 mesh) was produced from Qingdao Ocean Chemical Factory, China. ODS (50 μm) was obtained

from YMC Co. LTD., Kyoto, Japan. Sephadex LH-20 was produced by GE Healthcare. Preparative HPLC separations were performed on a SEP system (Beijing Sepuruisi scientific Co., Ltd., China) equipped with a variable-wavelength UV detector, using a YMC-Pack ODS-A column (250 × 20 mm, 5μm). Chemical reagents for isolation were of analytical grade and purchased from Tianjin Siyou Co., Ltd., China. Biological reagents were from Sigma Company. Human heptocellular (HepG2) and breast (MCF-7) cell lines were from Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, China.

Plant material

The fruits of *S. emodi* were collected in Deqin, Yunnan province, China, in September 2013, and authenticated by Prof. Cheng-Ming Dong at School of Pharmacy, Henan University of Traditional Chinese Medicine, where A voucher specimen (SE 20130929) was deposited.

Extraction and isolation

The dried and powdered fruits of *S. emodi* (9.1 kg) were refluxed with 95% EtOH (3 × 20 L). The filtrate was concentrated under reduced pressure to yield a dark brown residue (1.6 kg). The residue was suspended in water (3.2 L) and partitioned with petroleum ether (PE, 3.2 L × 3), CH_2Cl_2 (3.2 L × 3), EtOAc (3.2 L × 3), and *n*-BuOH (3.2 L × 3), successively. The EtOAc extract (142.71 g) was separated into sixteen fractions E1–16 by silica gel column chromatography (CC, 100 × 10 cm) with a gradient of PE (60–90 □)–acetone (v/v 100 : 0, 100 : 5, 100 : 7, 100 : 10, 100 : 30, 100 : 50, 100 : 70, 100 : 100, 100 : 200, 0 : 100) based on TLC monitoring. Fraction E2 (4.51 g) was subjected to Sephadex LH-20 CC (70 × 2.5 cm) eluted by methanol to yield subfractions E2–1~E2–3. Sub-fraction E2–1 (1.75 g) was further chromatographed over open ODS (40 × 2 cm) eluted with a gradient of methanol–H_2O (v/v 20 : 80, 60 : 40, 65 : 35, 70 : 30, 75 : 25) to **1** (13.8 mg), **2** (4.6 mg), **4** (5.1 mg), **5** (10.2 mg). Sub-fraction E2–3 (0.62 g) was further purified by silica gel CC (30 × 1 cm) eluted with PE–acetone (100 : 10) to give **22** (50.3 mg). Fraction E3 (3.49 g) was separated by open ODS (50 × 5 cm) eluted with a gradient of methanol–H_2O (v/v 20 : 80, 30 : 70, 40 : 60, 50 : 50) to give **17** (17.3 mg), **18** (43.8

mg), **19** (35.5 mg), and **21** (5.9 mg). Fraction E4 (4.87 g) was subjected to Sephadex LH-20 CC (70 × 2.5 cm) eluted by methanol to yield subfractions E4–1 and E4–2. Sub-fraction E4–1 (2.46 g) was further chromatographed over open ODS (35 × 3 cm) eluted with a gradient of methanol–H_2O (40 : 60, 70 : 30, 80 : 20) to afford **13** (70.4 mg). Sub-fraction E4–2 (2.18 g) was further submitted to silica gel CC (40 × 2 cm) eluted by PE–acetone (100 : 50) to give **20** (525.8 mg) and **24** (15.2 mg). Fraction E5 (6.30 g) was subjected to Sephadex LH-20 CC (100 × 4 cm) eluted by methanol to yield subfractions E5–1~ E5–3. Sub-fraction E5–1 (1.44 g) was further was applied to preparative HPLC eluted with methanol–H_2O (80 : 20) at a flow rate of 7 mL min^{-1} to give **8** (15.8 mg, t_R 21 min), **9** (3.1 mg, t_R 25 min), **11** (4.2 mg, t_R 33 min), **12** (5.7 mg, t_R 36 min), and **23** (25.2 mg, t_R 16 min). Sub-fraction E5–2 (2.57 g) was purified by silica gel CC (45 × 2 cm) eluted by PE–acetone (100:7, 100:10, 100:15, 100: 20) to yield **3** (7.2 mg), **6** (4.5 mg) and **7** (5.9 mg). Fraction E6 (3.58 g) was submitted to Sephadex LH-20 CC (60 × 2.5 cm) eluted by methanol to yield subfractions E6–1~E6–3. Sub-fraction E6–1 (1.49 g) was further subjected to open ODS (30 × 2.5 cm) eluted by methanol–H_2O (20 : 80, 50 : 50, 60 : 40, 70 : 30, 80 : 20) to afford sub-fraction E6–1–1~E6–1–3. Sub-fraction E6–1–2 was purified by preparative HPLC eluted with methanol–H_2O (80 : 20) at a flow rate of 7 mL min^{-1} to give **10** (2.8 mg, t_R 31 min). Sub-fraction E6–1–3 was subjected to by preparative HPLC eluted with methanol–H_2O (77 : 23) at a flow rate of 7 mL min^{-1} to give **14** (14.8 mg, t_R 22 min), **15** (11.5 mg, t_R 27 min), **16** (6.3 mg, t_R 34 min).

Sinoflavonoid C (1). yellow, amorphous powder; UV (MeOH) λmax (log ε) 263 (2.83), 344 (1.27) nm; IR (KBr) ν_{max} 3373, 2976, 2930, 1651, 1613, 1568, 1490, 1428, 1360, 1308, 1228, 1192 cm^{-1}; HR-ESI-MS (positive): *m*/*z* 475.1731 $[M + Na]^+$ (calcd for $C_{26}H_{28}O_7Na$, 475.1733); NMR data (DMSO-d_6), see Tables 1 and 2.

Sinoflavonoid D (2). yellow, amorphous powder; UV (MeOH) λmax (log ε) 262 (0.55), 327 (0.32) nm; IR (KBr) ν_{max} 3392, 2976, 2930, 1647, 1614, 1576, 1471, 1457, 1355, 1201 cm^{-1}; HR-ESI-MS (positive): *m*/*z* 475.1737 $[M + Na]^+$ (calcd for $C_{26}H_{28}O_7Na$, 475.1733); NMR data (DMSO-d_6), see Tables 1 and 2.

Sinoflavonoid E (3). yellow, amorphous powder; UV (MeOH) λmax (log ε) 263

(0.81), 344 (0.39) nm; IR (KBr) ν_{max} 3391, 2925, 2853, 1653, 1599, 1488, 1452, 1360, 1228, 1160 cm^{-1}; HR-ESI-MS (positive): *m*/*z* 439.1760 $[M + H]^+$ (calcd for $C_{25}H_{27}O_7$, 439.1757), *m*/*z* 461.1576 $[M + Na]^+$ (calcd for $C_{25}H_{26}O_7Na$, 461.1576); NMR data (DMSO-d_6), see Tables 1 and 2.

Sinoflavonoid F (4). yellow, amorphous powder; UV (MeOH) λmax (log ε) 260 (1.29), 339 (0.75) nm; IR (KBr) ν_{max} 3412, 2955, 2924, 2852, 1654, 1597, 1489, 1458, 1358, 1224, 1192, 1167 cm^{-1}; HR-ESI-MS (positive): *m*/*z* 385.1262 $[M + H]^+$ (calcd for $C_{21}H_{21}O_7$, 385.1287); NMR data (DMSO-d_6), see Tables 1 and 2.

Sinoflavonoid G (5). yellow, amorphous powder; UV (MeOH) λmax (log ε) 262 (0.28), 339 (0.12) nm; IR (KBr) ν_{max} 3429, 2954, 2925, 2852, 1652, 1605, 1572, 1459, 1374, 1335, 1304, 1230, 1160, 1091 cm^{-1}; HR-ESI-MS (positive): *m*/*z* 453.1910 $[M + H]^+$ (calcd for $C_{26}H_{29}O_7$, 453.1913); NMR data (DMSO-d_6), see Tables 1 and 2.

Sinoflavonoid H (6). yellow, amorphous powder; UV (MeOH) λmax (log ε) 265 (0.45), 343 (0.19) nm; IR (KBr) ν_{max} 3406, 2975, 2930, 2855, 1656, 1595, 1488, 1448, 1354, 1229, 1161 cm^{-1}; HR-ESI-MS (positive): *m*/*z* 491.1477 $[M + K]^+$ (calcd for $C_{26}H_{28}O_7K$, 491.1472); NMR data (DMSO-d_6), see Tables 1 and 2.

Sinoflavonoid I (7). yellow, amorphous powder; UV (MeOH) λmax (log ε) 255 (0.27), 327 (0.12) nm; IR (KBr) ν_{max} 3425, 2955, 2924, 2851, 1596, 1461, 1377, 1357, 1161, 1090 cm^{-1}; HR-ESI-MS (positive): *m*/*z* 453.1892 $[M + H]^+$ (calcd for $C_{26}H_{29}O_7$, 453.1913); NMR data (DMSO-d_6), see Tables 1 and 2.

Sinoflavonoid J (8). yellow, amorphous powder; UV (MeOH) λmax (log ε) 263 (2.89), 344 (1.42) nm; IR (KBr) ν_{max} 3395, 2958, 2925, 2853, 1650, 1611, 1592, 1569, 1497, 1451, 1362, 1294, 1158 cm^{-1}; HR-ESI-MS (positive): *m*/*z* 471.2024 $[M + H]^+$ (calcd for $C_{26}H_{31}O_8$, 471.2019); NMR data (DMSO-d_6), see Tables 1 and 2.

Sinoflavonoid K (9). yellow, amorphous powder; UV (MeOH) λmax (log ε) 264 (1.45), 342 (0.48) nm; IR (KBr) ν_{max} 3417, 2962, 2927, 2854, 1651, 1614, 1591, 1568, 1497, 1453, 1362, 1294, 1226, 1192, 1172 cm^{-1}; HR-ESI-MS (positive): *m*/*z* 509.1581 $[M + K]^+$ (calcd for $C_{26}H_{30}O_8K$, 509.1578); NMR data (DMSO-d_6), see Tables 1 and 2.

Sinoflavonoid L (10). yellow, amorphous powder; UV (MeOH) λmax (log ε) 263 (3.43), 344 (1.47) nm; IR (KBr) ν_{max} 3411, 2972, 2928, 2872, 1650, 1595, 1490, 1450, 1361, 1295, 1227, 1193, 1157 cm^{-1}; HR-ESI-MS (positive): *m*/*z* 493.1836 $[M + Na]^+$ (calcd for $C_{26}H_{30}O_8Na$, 493.1838); NMR data (DMSO-d_6), see Tables 1 and 2.

Sinoflavonoid M (11). yellow, amorphous powder; UV (MeOH) λmax (log ε) 264 (1.92), 337 (0.78) nm; IR (KBr) ν_{max} 3413, 2955, 2925, 2853, 1657, 1593, 1486, 1461, 1358, 1297, 1193, 1161 cm^{-1}; HR-ESI-MS (positive): *m*/*z* 471.2021 $[M + H]^+$ (calcd for $C_{26}H_{31}O_8$, 471.2019); NMR data (DMSO-d_6), see Tables 1 and 2.

Sinoflavonoid N (12). yellow, amorphous powder; UV (MeOH) λmax (log ε) 264 (0.79), 344 (0.35) nm; IR (KBr) ν_{max} 3392, 2974, 2930, 1652, 1595, 1488, 1451, 1356, 1229, 1161 cm^{-1}; HR-ESI-MS (positive): *m*/*z* 507.1993 $[M + Na]^+$ (calcd for $C_{27}H_{32}O_8Na$, 507.1995); NMR data (DMSO-d_6), see Tables 1 and 2.

Sinoflavonoid O (13). yellow, amorphous powder; UV (MeOH) λmax (log ε) 264 (0.78), 337 (0.31) nm; IR (KBr) ν_{max} 3406, 2974, 2934, 1656, 1594, 1489, 1449, 1358, 1274, 1192, 1161 cm^{-1}; HR-ESI-MS (positive): *m*/*z* 485.2157 $[M + H]^+$ (calcd for $C_{27}H_{33}O_8$, 485.2175); NMR data (DMSO-d_6), see Tables 1 and 2.

Cytotoxicity Asssays. The isolates were tested against MCF-7 and HepG2 cell lines, using an established MTT assay protocol.[9] Etoposide was used as the positive control.

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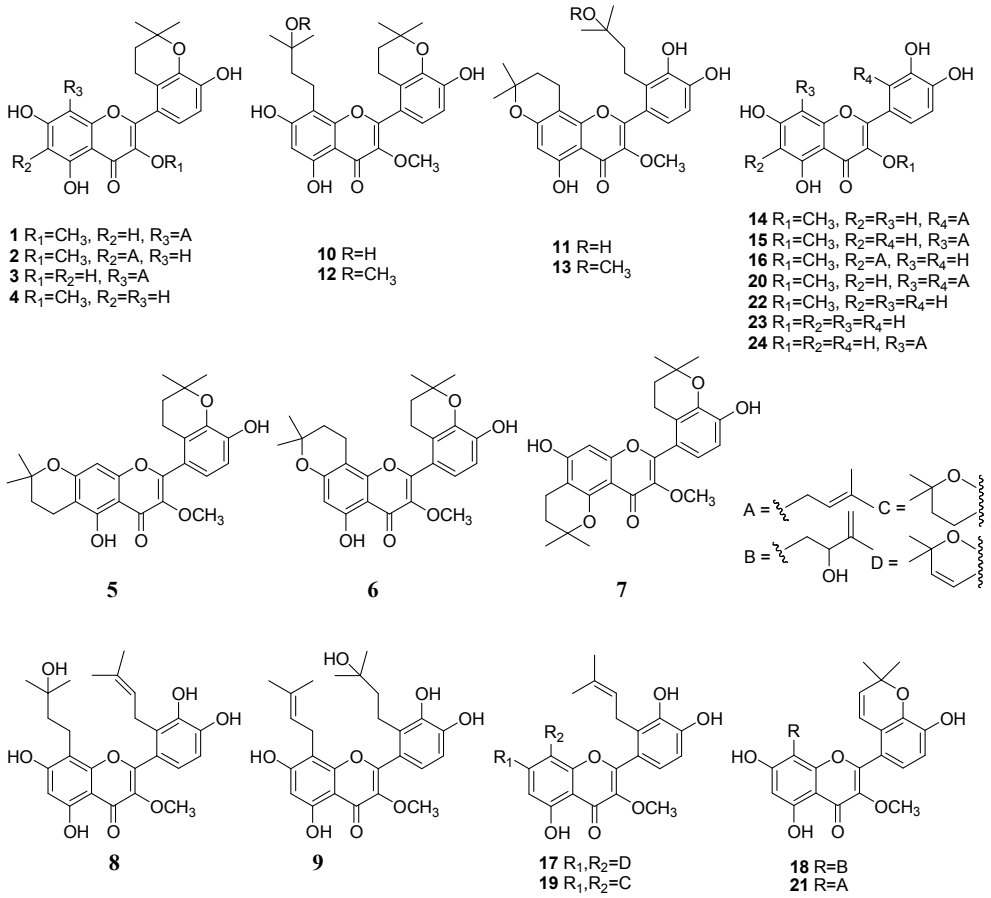

Fig. 1 Structures of compounds **1–24**

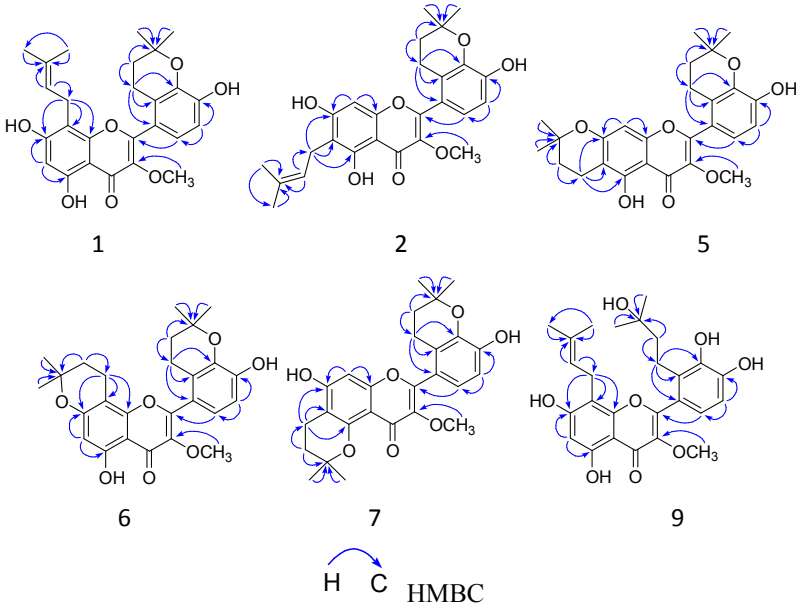

Fig. 2 Key HMBC correlations of compounds 1, 2, 5–7, 9

Table 1. ^{1}H NMR Spectroscopic Data (500 MHz, DMSO-d_6) of 1–13[a]

no.	1	2	3	4	5	6	7
6	6.30 s		6.28 s	6.20 s		6.16 s	
8		6.42 s		6.35 s	6.41 s		6.32 s
5′	6.73 d (8.5)	6.73 d (8.2)	6.72 d (8.2)	6.73 d (8.2)	6.73 d (8.2)	6.75 d (7.8)	6.70 d (8.2)
6′	6.85 d (8.5)	6.85 d (8.2)	6.86 d (8.2)	6.87 d (8.2)	6.87 d (8.2)	6.91 d (7.8)	6.79 d (8.2)
1″	3.23 d (6.9)	3.22 d (7.1)	3.25 d (7.0)	2.62 t (6.7)	2.61 t (6.6)	2.64 t (6.9)	2.54 t (6.9)
2″	5.04 t (6.9)	5.15 t (7.1)	5.06 t (7.0)	1.71 t (6.7)	1.81 t (6.6)	1.73 t (6.9)	1.74 t (6.9)
4″	1.48 s	1.71 s	1.52 s	1.30 s	1.30 s	1.29 s	1.31 s
5″	1.54 s	1.61 s	1.56 s	1.30 s	1.30 s	1.29 s	1.31 s
1‴	2.59 t (6.7)	2.60 t (6.7)	2.67 t (6.7)		2.61 t (6.6)	2.67 t (6.9)	2.57 t (6.5)
2‴	1.72 t (6.7)	1.71 t (6.7)	1.71 t (6.7)		1.70 t (6.6)	1.78 t (6.9)	1.71 t (6.5)
4‴	1.30 s	1.30 s	1.30 s		1.30 s	1.30 s	1.30 s
5‴	1.30 s	1.30 s	1.30 s		1.30 s	1.30 s	1.30 s
OCH_3	3.57 s	3.56 s		3.57 s	3.58 s	3.57 s	3.50 s
5-OH	12.58 s	12.89 s	12.45 s	12.63 s	12.98 s	12.43 s	
7-OH	10.79 s	10.87 s	10.68 s	10.87 s			10.59 s

no.	8	9	10	11	12	13
6	6.29 s	6.29 s	6.29 s	6.15 s	6.29 s	6.16 s
5′	6.74 d (8.2)	6.72 d (8.5)	6.74 d (8.2)	6.74 d (8.2)	6.73 d (8.2)	6.75 d (8.3)
6′	6.76 d (8.2)	6.74 d (8.5)	6.88 d (8.2)	6.76 d (8.2)	6.83 d (8.2)	6.78 d (8.3)
1″	2.56 m	3.21 d (7.2)	2.58 m	2.65 t (6.4)	2.55 m	2.62 t (6.6)
2″	1.45 m	5.02 t (7.2)	1.46 m	1.77 t (6.4)	1.50 m	1.77 t (6.6)
4″	1.00 s	1.44 s	1.04 s	1.29 s	1.03 s	1.29 s
5″	1.00 s	1.53 s	1.04 s	1.29 s	1.03 s	1.29 s
1‴	3.30 d (6.8)	2.54 m	2.68 t (6.6)	2.59 m	2.66 t (6.7)	2.50 m
2‴	4.98 t (6.8)	1.41 m	1.73 t (6.6)	1.54 m	1.72 t (6.7)	1.59 m
4‴	1.27 s	0.89 s	1.30 s	0.92 s	1.30 s	0.93 s
5‴	1.42 s	0.89 s	1.30 s	0.92 s	1.30 s	0.93 s
OCH_3	3.53 s	3.59 s	3.57 s	3.59 s	3.57 s	3.59 s
OCH_3					2.94 s	2.85 s
5-OH	12.58 s	12.58 s	12.64 s	12.45 s	12.59 s	12.43 s
7-OH	10.68 s	10.75 s	10.68 s		10.73 s	

[a]The coupling constants are in parentheses and reported in Hz; chemical shifts are given in ppm.

Table 2. ^{13}C NMR Spectroscopic Data (100 MHz, DMSO-d_6) of 1–13[a]

no.	1	2	3	4	5	6	7	8	9	10	11	12	13
2	158.8 s	158.06 s	149.5 s	158.3 s	158.2 s	158.7 s	154.6 s	158.7 s	158.8 s	158.5 s	158.7 s	158.4 s	159.5 s
3	139.1 s	138.8 s	136.3 s	138.9 s	138.7 s	139.2 s	140.7 s	138.5 s	138.7 s	138.7 s	139.2 s	138.7 s	139.3 s
4	178.6 s	178.0 s	176.5 s	178.0 s	178.2 s	178.2 s	172.0 s	178.3 s	178.3 s	178.3 s	178.2 s	178.3 s	178.2 s
5	159.4 s	158.13 s	158.5 s	161.4 s	158.5 s	158.4 s	157.0 s	159.5 s	159.5 s	158.8 s	159.4 s	158.9 s	158.7 s
6	98.5 d	110.7 s	97.7 d	98.6 d	104.3 s	99.1 d	105.0 s	98.2 d	98.2 d	98.2 d	99.1 d	98.1 d	99.2 d
7	161.9 s	161.9 s	161.1 s	164.2 s	159.8 s	159.5 s	159.8 s	161.6 s	161.4 s	161.6 s	159.8 s	161.7 s	159.7 s
8	106.3 s	93.0 d	105.5 s	93.8 d	94.5 d	100.0 s	93.2 d	107.2 s	105.8 s	107.6 s	99.8 s	106.8 s	99.6 s
9	154.5 s	154.6 s	154.1 s	156.8 s	154.4 s	153.9 s	154.0 s	154.2 s	154.2 s	154.0 s	153.9 s	154.1 s	153.8 s
10	105.0 s	104.4 s	103.5 s	104.6 s	104.5 s	105.3 s	107.6 s	104.6 s	104.6 s	104.6 s	105.3 s	104.5 s	105.3 s
1′	120.8 s	120.3 s	121.2 s	120.2 s	121.07 s	120.3 s	120.8 s	121.3 s	121.4 s	120.3 s	120.9 s	120.8 s	121.0 s
2′	121.2 s	121.1 s	121.4 s	121.1 s	120.2 s	121.4 s	120.9 s	127.8 s	129.3 s	120.9 s	129.2 s	121.3 s	128.8 s
3′	142.3 s	142.0 s	141.8 s	142.0 s	142.0 s	142.0 s	141.9 s	143.2 s	143.2 s	141.9 s	143.4 s	141.9 s	143.4 s
4′	148.5 s	148.2 s	147.7 s	148.2 s	148.2 s	148.3 s	147.6 s	146.9 s	146.7 s	148.1 s	147.9 s	148.7 s	147.9 s
5′	113.1 d	112.7 d	112.6 d	112.7 d	112.6 d	112.8 d	112.6 d	112.5 d	112.4 d	112.7 d	112.4 d	112.7 d	112.5 d
6′	121.5 d	121.0 d	121.0 d	121.06 d	121.14 d	121.2 d	120.8 d	121.09 d	120.6 d	121.4 d	121.0 d	120.4 d	120.9 d
1′′	21.4 t	20.9 t	21.0 t	20.1 t	15.7 t	15.5 t	16.7 t	17.3 t	21.2 t	17.3 t	15.8 t	16.5 t	15.8 t
2′′	122.6 d	122.1 d	122.4 d	31.8 t	30.9 t	31.8 t	30.8 t	42.9 t	121.9 d	43.0 t	30.9 t	38.2 t	30.8 t
3′′	131.3 s	130.7 s	130.8 s	73.9 s	76.2 s	76.3 s	74.8 s	68.8 s	130.8 s	68.7 s	76.2 s	73.6 s	76.2 s
4′′	17.8 q	17.7 q	17.5 q	26.5 q	26.3 q	26.3 q	26.41 q	28.9 q	17.4 q	28.9 q	26.2 q	24.8 q	26.1 q
5′′	25.8 q	25.5 q	25.4 q	26.5 q	26.3 q	26.3 q	26.41 q	28.9 q	25.4 q	28.9 q	26.2 q	24.8 q	26.1 q
1′′′	20.5 t	20.1 t	20.3 t		20.1 t	20.5 t	20.1 t	25.6 t	22.3 t	20.3 t	22.6 t	20.3 t	21.9 t
2′′′	32.2 t	31.8 t	31.9 t		31.8 t	31.8 t	31.8 t	122.9 d	43.5 t	31.8 t	43.3 t	31.8 t	39.9 t
3′′′	74.3 s	73.9 s	73.8 s		73.9 s	73.4 s	73.8 s	130.2 s	68.6 s	73.9 s	68.5 s	73.9 s	73.5 s
4′′′	26.8 q	26.4 q	26.4 q		26.5 q	26.5 q	26.42 q	17.3 q	28.7 q	26.5 q	28.8 q	26.4 q	24.4 q
5′′′	26.8 q	26.4 q	26.4 q		26.5 q	26.5 q	26.42 q	25.3 q	28.7 q	26.5 q	28.8 q	26.4 q	24.4 q
OCH_3	60.6 q	60.2 q		60.2 q	60.2 q	60.3 q	59.8 q	59.8 q	60.0 q	60.2 q	60.0 q	60.2 q	60.0 q
OCH_3												48.3 q	48.2 q

[a]The assignments were based on HSQC and HMBC spectra.

Table 3. Cytotoxicities of 1–24 against MCF-7 and HepG2 cell lines (IC_{50}, μM)

Compound	MCF-7	HepG2	Compound	MCF-7	HepG2
1	>100	>100	**14**	9.50±0.64	2.46±0.11
2	>100	>100	**15**	59.5±4.6	78.2±5.3
3	76.5±5.9	83.2±6.6	**16**	>100	>100
4	>100	>100	**17**	>100	>100
5	>100	>100	**18**	24.7±2.2	43.5±3.6
6	42.6±2.8	33.9±2.7	**19**	31.0±2.9	50.9±4.4
7	53.7±4.2	38.1±3.5	**20**	>100	>100
8	22.6±1.7	51.5±3.8	**21**	>100	>100
9	28.3±2.5	35.4±2.6	**22**	3.14±0.28	2.08±0.16
10	33.4±3.0	18.2±2.5	**23**	30.3±2.7	21.8±1.5
11	58.3±4.3	>100	**24**	>100	>100
12	52.7±4.1	47.0±3.3	etoposide	3.17±0.25	0.48±0.03
13	47.5±3.9	20.3±1.4			

Thirteen new prenylated flavonoids were isolated from *Sinopodophyllum emodi* together with eleven known analogues. Compound 22 exhibited the most potent cytotoxicity against MCF-7 and HepG2 cell lines.