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Rare prenylated isoflavonoids from the young twigs of *Millettia extensa* and their cytotoxic activities†

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Three new isoflavonoids, millexatins N–P (**1–3**), along with seven known compounds (**6–10**), were isolated from the acetone extract of the young twigs of *Millettia extensa*. The structures were characterized by NMR spectroscopic and mass spectrometric analyses. Millexatin N (**1**) is an unusual geminal diisoprenylated isoflavone with a modified ring A. Millexatin P (**3**) is an unusual isoflavone with a cyclohexyl substituent on ring B, which is extremely rare in nature. The isolated metabolites (**1**, **2**, and **6–10**) were evaluated for cytotoxicities against MDA-MB231, Huh-7, KKU-100 and normal human dermal fibroblast (NHDF) cell lines. Only compounds **1**, **6** and **8** showed cytotoxicities against all cell lines with IC_{50} values ranging from 13.9 to 30.9 μM.

Introduction

Isoflavonoids possess a 3-phenyl-chromen-4-one scaffold found abundantly in the family Leguminosae, and many of them are considered for promising pharmacological properties and health benefits on multitarget tissues.[1–3] Especially the prenylated forms have more potential to be developed and utilized for medicinal purposes and as lead compounds due to lipophilicity, as compared to nonprenylated forms, leading to high affinity with cell membranes and significant pharmacological activity.[1]

The genus *Millettia*, belonging to the family Fabaceae, is widespread predominantly in the subtropical and tropical regions of Africa, Asia, and Australasia.[4] Many species of this genus such as *M. extensa*, *M. conraui*, *M. brandisiana*, and *M. auriculata* have been used for the treatment of infected wound, tonic, skin infection, cough, boils, sores, insecticide, haematonic, and fish poison.[5–8] Various types of compounds have been isolated from this genus, including isoflavonoids, chalcones, benzofuran-chalcones, rotenoids, and flavonoids,[9–15] some of

which showed valuable anti-estrogenic,[16] anti-inflammatory,[12] antibacterial,[11] antiplasmodial,[17,18] NAD(P)H quinine oxidoreductase 1-inducing,[14] inhibitory effects on NLRP3 inflammasome activation,[19] and cytotoxic[20] activities.

Millettia extensa (Benth.) Baker, named "Kao Khruea" in Thai, is widely distributed in most tropical areas and it is also mostly found in the northern part of Thailand. As a traditional medicinal plant, the barks and roots have been used for the treatment of sprains, scabies, contraceptive, and protective medicine for women after childbirth.[21] Previous phytochemical studies into *M. extensa* have revealed prenylated isoflavonoids as one of the major classes of bioactive compounds with antibacterial and anti-inflammatory activities.[11,12] With the aim for searching for bioactive constituents from the medicinal plants growing in northern region of Thailand,[22] the acetone extract of

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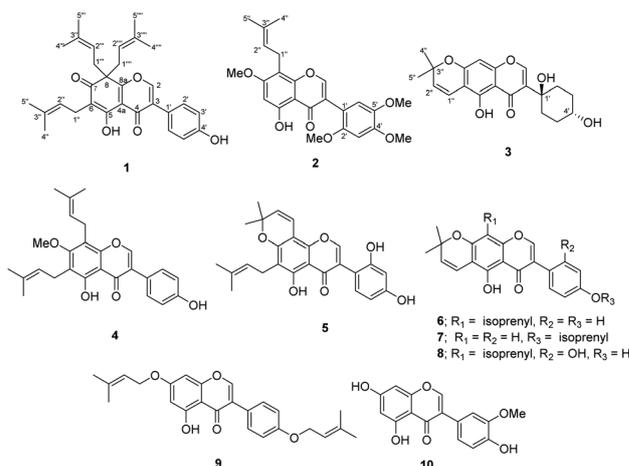
† Electronic supplementary information (ESI) available: HRESIMS, NMR spectra of compounds **1–3**. See DOI: https://doi.org/10.1039/d2ra05950a

Fig. 1 Structures of compounds **1–10**.

the stem barks of *M. extensa* was investigated. Three new iso-flavonoids (**1–3**) together with seven known compounds (**4–10**) (Fig. 1) and cytotoxic activities against human cancer cell lines of some isolated compounds are described in this paper.

Experimental

General experimental procedures

Optical rotation was obtained on a JASCO P-2000 polarimeter in MeOH. The UV spectra were recorded with a PerkinElmer UV-vis spectrophotometer, whereas the IR spectra were obtained using a PerkinElmer FTS FT-IR spectrophotometer. The Bruker Ultrashield 500 MHz NMR spectrometer was used to measure the 1D and 2D NMR spectra of the isolated compounds. Chemical shifts (δ) are expressed in ppm with CDCl$_3$ (δ_H 7.24 and δ_C 77.0) using TMS as an internal reference, reference to the solvent signals. A MicroTOF, Bruker Daltonics mass spectrometer was employed to acquire HRESIMS spectra. Column chromatography (CC) was performed on silica gel 60H (5–40 μm), silica gel 100 (63–200 μm) and Sephadex LH-20 (Pharmacia Fine Chemical Co., Ltd., Sweden). Semipreparative HPLC separations were performed on a Waters 626 liquid chromatography system equipped with a Grace C18 column (Econosil C$_{18}$, 10 μm, 10.0 i.d. × 250 mm) and a Waters 486 Tunable Absorbance detector (Waters, USA). Fractions obtained from CC were monitored by TLC using precoated plates of silica gel 60 F$_{254}$ (Merck).

Plant material

The young twigs of *Millettia extensa* (Benth.) Baker, were collected in August 2021 at Maeka, Phayao Province, Thailand (GPS: 19°01′32.7″N 99°53′24.2″E) and were authenticated by Mr Martin van de Bult, Doi Tung Development Project. A voucher specimen (UP-CNP002) was deposited at the Chemistry of Natural Products for Sustainability Laboratory, School of Science, University of Phayao.

Extraction and isolation

The air-dried and ground young twigs of *M. extensa* (1.8 kg) were extracted by percolation with acetone (4 L) at room temperature (3 days × 2), and the solvent was evaporated under reduced pressure to give acetone extract (236.4 g). The crude extract was subjected to silica gel column chromatography (CC), eluting with 100% hexanes to 100% acetone, to provide seven fractions (A–G). Fraction A (2.5 g) was further purified through Sephadex LH-20 (MeOH/CH$_2$Cl$_2$, 1 : 1, v/v) to afford five subfractions (A1–A5). Subfraction A4 (840.4 mg) was subjected on silica gel CC, eluting with acetone–hexanes (1 : 19, v/v) to yield compounds **7** (8.2 mg) and **9** (7.7 mg). The separation of fraction C (17.4 g) by silica gel CC, eluting with 100% CH$_2$Cl$_2$ and an increasing polarity with MeOH, afforded five subfractions (C1–C5). Subfraction C5 (5.3 g) was purified by Sephadex LH-20 (100% MeOH), following purified by silica gel CC (acetone–hexanes, 1 : 4, v/v) to obtain five subfractions (C5a–C5e). Subfraction C5b (150.5 mg) was chromatographed on a silica gel CC with MeOH–CH$_2$Cl$_2$ (1 : 99, v/v) to yield compounds **1** (9.1 mg), **2** (5.9 mg), **4**

(2.6 mg) and **6** (8.0 mg). Fraction E (11.5 g) was loaded to silica gel CC eluting with a MeOH–CH$_2$Cl$_2$ gradient solvent system (0 : 1 to 1 : 9, v/v) and nine subfractions were obtained (E1–E9). Subfraction E2 (1.7 g) was separated by Sephadex LH-20 with 100% MeOH as eluent to afford five subfractions (E2a–E2e). Recrystallization of subfraction E2b with acetone–hexanes (1 : 4, v/v) gave compound **10** (3.1 mg). Subfraction E2c (87.4 mg) was achieved by silica gel CC eluting with acetone–hexanes (1 : 4, v/v) to yield compounds **5** (2.8 mg) and **8** (6.7 mg). Purification of fraction F (1.1 g) by Sephadex LH-20 eluting with MeOH and subjected to silica gel CC eluting with MeOH–CH$_2$Cl$_2$ (1 : 49, v/v) yielded five subfractions (F1–F5). Subfraction F4 (15.2 mg) was further purified by semipreparative C$_{18}$ HPLC with MeOH–H$_2$O (75 : 25, v/v) to yield compound **3** (2.1 mg, t_R 13.2).

Millexatin N (1). Yellow viscous oil; UV (MeOH) λ_{max} (log ε): 201 (4.10), 260 (3.64), 355 (3.10) nm; IR (neat) ν_{max} 3304, 2955, 1661, 1548, 1426, 1401 cm^{-1}; ^{1}H NMR (500 MHz, CDCl$_3$) and ^{13}C NMR (125 MHz, CDCl$_3$) spectra, Table 1; HRESIMS m/z 497.2299 [M + Na]$^+$ (calcd for [C$_{30}$H$_{34}$O$_5$Na]$^+$, 497.2298).

Millexatin O (2). Yellow viscous oil; UV (MeOH) λ_{max} (log ε): 205 (4.15), 269 (4.11), 330 (3.12) nm; IR (neat) ν_{max} 3332, 2958, 1706, 1635, 1589, 1435, 1252 cm^{-1}; ^{1}H NMR (500 MHz, CDCl$_3$) and ^{13}C NMR (125 MHz, CDCl$_3$) spectra, Table 1; HRESIMS m/z 449.1578 [M + Na]$^+$ (calcd for [C$_{24}$H$_{26}$O$_7$Na]$^+$, 449.1576).

Millexatin P (3). Yellow viscous oil; $[\alpha]_D^{27}$ + 0.20 (c 0.05, MeOH); UV (MeOH) λ_{max} (log ε): 202 (4.11), 268 (3.65), 310 (3.22) nm; IR (neat) ν_{max} 3430, 2970, 1665, 1589, 1430, 1254, cm^{-1}; ^{1}H NMR (500 MHz, CDCl$_3$) and ^{13}C NMR (125 MHz, CDCl$_3$) spectra, Table 1; HRESIMS m/z 359.1493 [M + H]$^+$ (calcd for [C$_{20}$H$_{23}$O$_6$]$^+$, 359.1489).

Cell lines and culture

Human CCA cell lines, namely KKU-100 (Japanese Collection of Research Bioresources Cell Bank (JCRB), National Institute of Biomedical Innovation, Japan), MDA-MB-231 and Huh-7 (ATCC, American Type Culture Collection), and normal human dermal fibroblast (PromoCell, Germany) were cultured in Dulbecco's modified Eagle's medium and Ham's F-12 nutrient mixture (DMEM/F12; Gibco, Thermo Fisher Scientific) supplemented with 10% fetal bovine serum (FBS; Gibco, Thermo Fisher Scientific) and penicillin (100 U mL^{-1})–streptomycin (100 μg mL^{-1}) at 37 °C in a humidified atmosphere of 5% CO$_2$. The cells were sub-cultured twice per week by following the standard trypsinization protocol.

Cytotoxicity assay

The procedure for the cytotoxic assay was assessed against KKU-100, MDA-MB-231, Huh-7, and normal human dermal fibroblast (NHDF) cell lines by the MTT method. Cell lines (5 × 10^3 cells per well) were seeded in a 96-well plate for 24 hours. Various tested compound concentrations were added to the 96-well plate and incubated for 24 h. Ten microliters of MTT solution (5 mg mL^{-1}) were added and incubated for 2 h. Then, cell supernatants were removed, and DMSO was added to dissolve the formazan product. A microplate spectrophotometer measured light absorbent at 540 nm. The IC$_{50}$ was calculated by

Table 1 ¹H (500 MHz) and ¹³C NMR (125 MHz) data for compounds 1–3

No.	Millexatin N (1)[a]		Millexatin O (2)[a]		Millexatin P (3)[a]	
	δ_C, type	δ_H, mult. (J in Hz)	δ_C, type	δ_H, mult. (J in Hz)	δ_C, type	δ_H, mult. (J in Hz)
2	152.6, CH	8.00, s	154.9, CH	7.95, s	151.7, CH	7.86, s
3	128.8, C		119.7, C		127.4, C	
4	178.9, C		179.1, C		183.0, C	
4a	114.6, C		106.5, C		106.0, C	
5	164.1, C		161.0, C		152.1, C	
6	115.4, C		95.2, CH	6.44, s	101.1, C	
7	195.3, C		162.4, C		159.9, C	
8	57.0, C		107.9, C		100.4, CH	6.29, s
8a	174.2, C		154.6, C		162.0, C	
1′	121.4, C		111.0, C		71.0, C	
2′	130.1, CH	7.40, d (8.7)	150.1, C		29.8, CH₂	2.15, m / 1.91, m
3′	116.0, CH	6.94, d (8.7)	98.2, CH	6.66, s	28.2, CH₂	2.12, m / 1.64, m
4′	157.2, C		152.1, C		65.6, CH	4.16, brs
5′	116.0, CH	6.94, d (8.7)	143.1, C		28.2, CH₂	2.12, m / 1.64, m
6′	130.4, CH	7.40, d (8.7)	115.1, CH	6.91, s	29.8, CH₂	2.15, m / 1.91, m
1″	21.0, CH₂	3.09, d (6.8)	21.5, CH₂	3.44, d (7.2)	114.4, CH	6.66, d (10.1)
2″	121.9, CH	5.07, brt (6.8)	122.0, CH	5.20, brt (7.2)	127.5, CH	5.59, d (10.1)
3″	131.9, C		132.6, C		78.3, C	
4″	18.0, CH₃	1.73, s	17.8, CH₃	1.82, s	28.3, CH₃	1.49, s
5″	26.0, CH₃	1.65, s	25.8, CH₃	1.70, s	28.3, CH₃	1.49, s
1‴/1⁗	38.7, CH₂	2.85, dd (13.9, 7.1) / 2.62, dd (13.9, 7.1)				
2‴/2⁗	117.1, CH	4.69, t (7.1)				
3‴/3⁗	136.0, C					
4‴/4⁗	17.9, CH₃	1.54, s				
5‴/5⁗	25.9, CH₃	1.50, s				
OH-5		13.13, s		12.97, s		13.59, s
OMe-7			56.1, CH₃	3.93, s		
OMe-2′			56.2, CH₃	3.97, s		
OMe-4′			56.9, CH₃	3.82, s		
OMe-5′			56.6, CH₃	3.88, s		

[a] NMR data were recorded in chloroform-*d*.

using GraphPad Prism version 8. Doxorubicin (Sigma) and 0.75% DMSO (RCI Labscan) were used as a positive and negative control.

Results and discussion

The air-dried and ground young twigs of *M. extensa* were extracted with acetone to afford a dark green crude extract. Purification of this extract by repeated chromatographic separation gave three undescribed isoflavones (1–3), along with seven known compounds that were identified as millewanin F (4),[23] millexatin F (5),[11] scandenone (6),[11] 2′-deoxyisoauriculatin (7),[24] auriculatin (8),[25] 7,4′-di-*O*-prenylgenistein (9),[26] and 3′-methylorobol (10)[27] upon comparison of their physical and spectroscopic data with published values.

Compound 1 was obtained as yellow viscous oil. Its molecular formula was deduced as $C_{30}H_{34}O_5$ on the basis of the

HRESIMS ion peak at *m/z* 497.2299 [M + H]⁺ (calcd for $[C_{30}H_{34}O_5Na]^+$, 497.2298), which accounted for 14 degrees of unsaturation. The IR spectrum displayed absorptions due to hydroxy group (3304 cm⁻¹) and conjugated carbonyl (1661 cm⁻¹) functionalities. Its UV spectrum at λ_{max} 260 and 355 nm were consistent with an isoflavone chromophore that was corroborated with a singlet at δ_H 8.00 (H-2) in the ¹H NMR spectrum, and the ¹³C NMR signals at δ_C 152.6 (C-2), 128.8 (C-3), and 178.9 (C-4). Analysis of the ¹³C NMR data (Table 1) of 1 displayed 30 carbon resonances, which were classified by their chemical shifts and HSQC spectrum as six methyls [δ_C 25.7, 25.9 (2), 17.7 and 17.9 (2)], eight sp² methines [δ_C 152.6, 130.4 (2), 121.9, 117.1 (2) and 116.0 (2)], three sp³ methylenes [δ_C 38.7 (2) and 21.0], 11 non-hydrogenated carbons [δ_C 174.2, 164.1, 157.2, 136.0 (2), 131.9, 128.8, 121.4, 115.4, 114.6, and 57.0], and two conjugated carbonyl carbons (δ_C 195.3 and 178.9). In the ¹H NMR spectrum (Table 1) of 1, a downfield signal at δ_H 13.13 (1H,

s) was assigned to a hydrogen-bonded hydroxy group at C-5. The ^1H and ^{13}C NMR signals showed resonances for an isoprenyl unit [δ_H/δ_C 5.07 (1H, brt, $J = 6.8$ Hz, H-2″)/121.9, 3.09 (2H, d, $J = 6.8$ Hz, H$_2$-1″)/21.0, 1.73 (3H, s, H$_3$-4″)/18.0, 1.65 (3H, s, H$_3$-5′)/26.0, and δ_C 131.9 (C-3″)] and a geminal diisoprenyl group [δ_H/δ_C 4.69 (2H, t, $J = 7.1$ Hz, H-2‴, H-2‴′)/117.1, 2.85 (2H, dd, $J = 13.9, 7.1$ Hz, H$_2$-1‴a, H$_2$-1‴′a)/38.7, 2.62 (2H, dd, $J = 13.9, 7.1$ Hz, H$_2$-1‴b, H$_2$-1‴′b), 1.54 (6H, s, H$_3$-4‴, H$_3$-4‴′)/17.9, 1.50 (6H, s, H$_3$-5‴, H$_3$-5‴′)/25.9, and δ_C 136.0 (C-3‴/C-3‴′)]. These spectroscopic data revealed that it is structurally closely related to millexatin A, an isoflavone that was isolated from the stems of *M. extensa*.[11] The major difference found was that a set of ABC spin-coupled aromatic protons of millexatin A was replaced with the two doublet resonances for the *para*-aromatic protons on the B ring [δ_H 7.40 (2H, d, $J = 8.7$ Hz, H-2′, H-6′), and 6.94 (2H, d, $J = 8.7$ Hz, H-3′, H-5′)]. The HMBC correlation (Fig. 2) of the methine proton H-2′/H-6′ with C-3, C-1′, C-3′ and C-4′ allowed the assignments of H-2′/H-6′, and H-3′/H-5′ of ring B. The long-range ^1H–^{13}C correlations from OH-5 (δ_H 13.13) to C-4a, C-5, C-6, and from H$_2$-1″ (δ_H 3.09) to C-5, C-6 and C-7 suggested the location of an isoprenyl unit at C-6 and a conjugated ketone carbonyl group at C-7. The HMBC correlations observed between the methylene protons H$_2$-1‴/H$_2$-1‴′ (δ_H 2.85 and 2.62) and C-7, C-8 and C-8a confirmed the attachment of a geminal diisoprenyl group at C-8 (Fig. 2). On the basis of these data, the structure of compound **1**, which features an unusual isoflavone with a geminal diisoprenyl unit on a modified ring A, was characterized as millexatin N.

Compound **2** was isolated as a yellow viscous oil and exhibited a [M + Na]⁺ ion peak at *m/z* 449.1578 (calcd. for [C$_{24}$H$_{26}$O$_7$Na]⁺, 449.1576) in the HRESIMS analysis, consistent with a molecular formula of C$_{24}$H$_{26}$O$_7$. The IR and UV spectra were similar to those of **1**, suggesting the presence of an isoflavone skeleton. The NMR spectroscopic data (Table 1) indicated resonances typical of an isoprenyl moiety [δ_H/δ_C 5.20 (1H, brt, $J = 7.2$ Hz, H-2″)/122.0, 3.44 (2H, d, $J = 7.2$ Hz, H$_2$-1″)/21.5, 1.82 (3H, s, H$_3$-4″)/17.8, 1.70 (3H, s, H$_3$-5″)/25.8, and δ_C 132.6(C-3″)], three aromatic protons [δ_H/δ_C 6.91 (1H, s, H-6′)/115.1, 6.66 (1H, s, H-3′)/98.2, and 6.44 (1H, s, H-6)/95.2], and four methoxy groups [δ_H/δ_C 3.97 (3H, s, OMe-2′)/56.2, 3.93 (3H, s, OMe-7′)/56.1, 3.88 (3H, s, OMe-5′)/56.6, and 3.82 (3H, s, OMe-4′)/56.9], along with a hydrogen-bonded hydroxy group [δ_H 12.97 (1H, s, 5-OH)]. In addition, a characteristic resonance for H-2 of an isoflavone at δ_H 7.95 (1H, s)/δ_C 154.9 was observed in the ^1H NMR spectrum, which was further confirmed by the key HMBC correlations (Fig. 2) from H-2 (δ_H 7.95) to C-3, C-4, C-8a and C-1′. The HMBC correlations of H-6 (δ_H 6.44) with C-4a, C-5, C-7 and C-8,
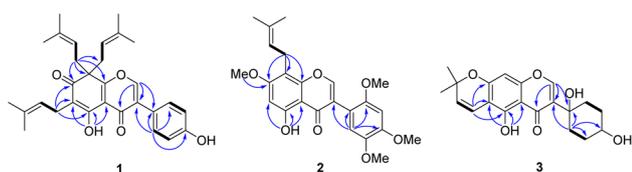
of H$_2$-1″ (δ_H 3.44) with C-7, C-8 and C-8a, and of 7-OMe (δ_H 3.93) with C-7 showed that an isoprenyl moiety and a methoxy group were attached to C-8 and C-7, respectively. Another methoxy groups at δ_H 3.97, 3.88, and 3.82 were placed at C-2′, C-5′ and C-4′, respectively, based on its HMBC correlations shown in Fig. 2 and the NOESY correlations of H-6′/OMe-5′ and OMe-2′/H-3′/OMe-4′. Therefore, the structure of compound **2** was established as shown and named millexatin O.

Compound **3**, isolated as a yellow viscous oil, was deduced to have a molecular formula of C$_{20}$H$_{22}$O$_6$ from its HRESIMS ion peak at *m/z* 359.1493 [M + H]⁺ (calcd. for [C$_{20}$H$_{23}$O$_6$]⁺, 359.1489), indicating 10 degrees of unsaturation. The IR absorptions implied the presence of hydroxy (3430 cm⁻¹) and carbonyl (1665 cm⁻¹) functionalities. The ^{13}C NMR and HMQC spectra (Table 1) revealed 20 carbon resonances for one carbonyl carbon (δ_C 183.0), eight non-hydrogenated carbons, of which one was a sp³ oxygenated carbon (δ_C 71.0), four sp² methines, including one oxygenated carbon (δ_C 151.7), four methylenes, and two methyls. Comparison of its 1D NMR data of **3** (Table 1) with compound **7** (ref. 24) suggested that these two compounds shared the same isoflavone skeleton with a hydrogen-bonded hydroxy group [δ_H 13.59 (1H, s, OH-5)], an olefinic proton [δ_H 7.86 (1H, s, H-2)/δ_C 151.7], an aromatic proton [δ_H 6.29 (1H, s, H-8)/δ_C 100.4], and a 1,1-dimethylallyl group [δ_H/δ_C 6.66 (1H, d, $J = 10.1$ Hz, H-1″)/114.4, 6.59 (1H, d, $J = 10.1$ Hz, H-2″)/127.5, 1.49 (6H, s, H$_3$-4″ and H$_3$-5″)/28.3, and δ_C 78.3 (C-3″)]. The difference between them was the absence of an isoprenyl group and four aromatic protons of ring B. Compound **3** displayed signals for 4-hydroxycyclohexyl moiety [δ_H/δ_C 4.16 (1H, brs, H-4′)/65.6, 2.15 (2H, m, H$_2$-2′a, H-6′a)/29.8, 2.12 (2H, m, H$_2$-3′a, H-5′a)/28.2, 1.91 (2H, m, H$_2$-2′b, H-6′b) and 1.64 (2H, m, H$_2$-3′b, H-5′b), and δ_C 71.0 (C-1′)] instead of the coupled aromatic protons in **7**. In the HMBC spectrum of **3** (Fig. 2), the correlations from H$_2$-2′/H$_2$-6′ to C-3, C-1′ and C-4′, from H-2 to C-4, C-8a, C-3 and C-1′ and the low field chemical shift of C-1′ at δ_C 71.0 indicated that hydroxy group was attached at C-1′ and 4-hydroxycyclohexyl moiety at C-3. These spectroscopic data were similar to those of 2-(*trans*-1,4-dihydroxy-2-cyclohexenyl)-5-hydroxy7-methoxychromone previously isolated from the fern of *Phegopteris connectilis*.[28] Additionally, the HMBC correlations of OH-5 (δ_H 13.59) and H-1″ (δ_H 6.66) with C-5 confirmed a 1,1-dimethylallyl group at C-6 and C-7. The relative configuration of **3** was established by analysis of NOESY data (Fig. 3) and ^1H–^1H couplings. The small J value ($J = <1$ Hz) between H-3′/H-4′ and H-4′/H-5′ and the cross-peaks of H-4′/H − 3′$_{ax}$(H − 5′$_{ax}$) and H-4′/H − 3′$_{eq}$(H − 5′$_{eq}$) in the NOESY spectrum indicated that the hydroxy group at C-4′ was α-axially oriented. In addition, the NOESY correlations of H-2 with H − 2′$_{ax}$ and H − 2′$_{eq}$, and of H − 2′$_{ax}$ with H − 3′$_{eq}$ and H − 2′$_{eq}$

Fig. 2 Selected $^1H \rightarrow {^{13}}C$ HMBC (blue arrows) and COSY (bold line) correlations of **1–3**.

Fig. 3 Key NOESY cross-peaks (blue) and coupling constants (red) of **3**.

Table 2 Cytotoxic activities of compounds **1**, **2**, **6–10**

Compound	Cell lines (µM)			
	MDA-MB231	Huh-7	KKU-100	NHDF fibroblast
1	15.4	14.8	22.4	17.5
2	>100	>100	>100	>100
6	13.9	16.3	21.6	18.2
7	>100	>100	>100	>100
8	15.3	26.7	23.8	30.9
9	>100	>100	>100	>100
10	>100	>100	>100	>100
Doxorubicin[a]	3.1	NT[b]	1.9	5.0

[a] Positive control. [b] Not tested.

implied that the hydroxy group at C-1′ was in an β-axial direction. These were in good agreement with the relative configurations at C-1′ and C-4′ to that of related compound in the literature.[28] The observation of a specific rotation value ($[\alpha]_D^{27}$ + 0.20 (*c* 0.05, MeOH)) and the lack of a Cotton effect was observed, suggesting **3** to be a racemate. Thus, the structure of compound **3** was named millexatin P.

Most of the isolated isoflavones (**1**, **2**, **6–10**) were tested for their cytotoxic activities against breast (MDA-MB231), hepatocellular carcinoma (Huh-7), cholangiocarcinoma (KKU-100) and normal human dermal fibroblasts (NHDF) using the MTT assay, with doxorubicin as the positive control (Table 2). Compounds **1**, **6** and **8** exhibited cytotoxic activity against all the cell lines with IC$_{50}$ values ranging from 13.9 to 30.9 µM. Compound **1** was found to be the best cytotoxic effect against Huh-7 cell line. However, this compound was relatively cytotoxic. Compounds **2**, **7**, **9** and **10** were inactive toward all (IC$_{50}$ > 100 µM). It is interesting to note that compound **6** with an isoprenyl moiety at C-8 had better activity than **7**, which lacked this substituent.

Conclusions

In conclusion, two undescribed modified isoflavones (**1** and **3**) and one undescribed methylated isoflavone (**2**) together with seven known compounds (**4–10**) were isolated from the young twigs of *M. extensa*. Compounds **1** is a rare isoflavone with a geminal diisoprenyl unit on a modified ring A, which compound **3** is unusual isoflavone with saturated ring B. Several modified isoflavones were also isolated from the genus *Millettia*.[15,29,30] Moreover, compounds **1**, **6** and **8** were found to have cytotoxic effect against MDA-MB231, Huh-7, KKU-100 and normal human dermal fibroblasts cell lines.

Author contributions

S. Cheenpracha, contributed to developing the concept, experimental work, formal analysis and writing–original draft; R. Chokchaisiri, S. Laphookhieo, T. Limtharakul, contributed to experimental design and writing–review & editing; C. Thepmalee, contributed to experimental design, writing–review & editing. All authors have read and approved the final version of the manuscript.

Conflicts of interest

There are no conflicts to declare.

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