UNIVERSITY
OF WOLLONGONG
AUSTRALIA

University of Wollongong
Research Online

Faculty of Science, Medicine and Health - Papers:
Part B

Faculty of Science, Medicine and Health

2018

Antibacterial Prenylated Isoflavonoids from the Stems of *Millettia extensa*

Achara Raksat

Mae Fah Luang University

Wisanu Maneerat

Mae Fah Luang University

Raymond J. Andersen

University of British Columbia

Stephen G. Pyne

University of Wollongong, spyne@uow.edu.au

Surat Laphookhieo

Mae Fah Luang University, surat@uow.edu.au

Publication Details

Raksat, A., Maneerat, W., Andersen, R. J., Pyne, S. G. & Laphookhieo, S. (2018). Antibacterial Prenylated Isoflavonoids from the Stems of *Millettia extensa*. *Journal of Natural Products*, 81 (8), 1835-1840.

Research Online is the open access institutional repository for the University of Wollongong. For further information contact the UOW Library:
research-pubs@uow.edu.au

Antibacterial Prenylated Isoflavonoids from the Stems of *Millettia extensa*

Abstract

The first phytochemical investigation of the stem extract of *Millettia extensa* resulted in the isolation and identification of six new isoflavones, millexatins A-F (1-6), together with 16 known compounds. The structures of these new compounds were determined on the basis of their spectroscopic data. Millexatin A (1) is a rare isoflavone containing three isoprenyl units on a modified A ring. Compounds 1, 6, 10, 11, and 14 displayed promising antibacterial activity with MIC values of 2-8 µg/mL.

Publication Details

Raksat, A., Maneerat, W., Andersen, R. J., Pyne, S. G. & Laphookhieo, S. (2018). Antibacterial Prenylated Isoflavonoids from the Stems of *Millettia extensa*. *Journal of Natural Products*, 81 (8), 1835-1840.

Antibacterial Prenylated Isoflavonoids from the Stems of *Millettia extensa*

Achara Raksat,^{†,‡} Wisanu Maneerat,^{†,‡} Raymond J. Andersen,[§] Stephen G. Pyne,^{||} and Surat
Laphookhieo^{*,†,‡}

[†] Center of Chemical Innovation for Sustainability (CIS), Mae Fah Luang University, Chiang
Rai 57100, Thailand

[‡] School of Science, Mae Fah Luang University, Chiang Rai 57100, Thailand

[§] Department of Chemistry and Department of Earth, Ocean & Atmospheric Sciences,
University of British Columbia, 2036 Main Mall, Vancouver, BC, Canada V6T 1Z1

^{||} School of Chemistry, University of Wollongong, Wollongong, New South Wales 2522,
Australia

ABSTRACT: The first phytochemical investigation of the stem extract of *Millettia extensa* resulted in the isolation and identification of six new isoflavones, millexatins A-F (**1-6**) together with 16 known compounds. The structures of these new compounds were determined on the basis of their spectroscopic data. Millexatin A (**1**) is a rare isoflavone containing three isoprenyl units on a modified A ring. Compounds **1**, **6**, **10**, **11**, and **14** displayed promising antibacterial activity with MIC values of 2-8 µg/mL.

The genus *Millettia* belongs to the family Leguminosae, which is well known to produce flavonoids, chalcones, rotenoids and isoflavonoids.¹⁻⁴ These types of compounds have been recognized to have cytotoxic,³⁻⁶ antimalarial,⁷⁻⁹ and antiinflammatory^{5,10,11} activities. Some species of *Millettia* have been utilized as traditional medicines to treat various diseases including dysmenorrhea, rheumatic pain, paralysis, snakebites, insect bites and inflammation.¹²⁻¹⁴ *Millettia extensa* (Benth.) Baker is a large climbing shrub that is distributed mainly in the northern part of Thailand. According to the information from the SciFinder Scholar database (Chemical Abstracts Service, Columbus, OH, USA), no phytochemical investigation or biological evaluation of this plant has been reported and its crude acetone extract showed good antibacterial activities. This information led us to investigate antibacterial compounds from this plant. Six new isoflavonoids (**1-6**) together with 16 known compounds and antibacterial activities of some isolated compounds are described in this paper.

RESULTS AND DISCUSSION

The extract of the stems of *M. extensa* was subjected to purification by repeated column chromatography which led to the isolation and elucidation of 19 isoflavones (**1-19**), two rotenoids (**20** and **21**), and a pterocarpan (**22**). By comparing their physical and spectroscopic data with published values, known compounds (**7-22**) were identified as 3'-methylobol (**7**),¹⁵ 7,4'-di-*O*-prenylgenistein (**8**),^{16,17} 5-hydroxy-7-methoxy-4'-*O*-(3-methylbut-2-enyl)isoflavone (**9**),¹⁸ auriculatin (**10**),^{19,20} scandenone (**11**),^{21,22} 5-*O*-methyl-4'-*O*-(3-methyl-2-butenyl)alpinumisoflavone (**12**),²³ elongatin (**13**),²⁴ auriculasin (**14**),^{20,21} 2'-*O*-methylisoauriculatin (**15**),²² 4'-methylalpinumisoflavone (**16**),²⁵ 2'-deoxyisoauriculatin (**17**),²² isoauriculasin (**18**),²⁰ isoauriculatin (**19**),^{20,26} (–)-sumatrol (**20**),¹⁹ (–)-6a,12a-dehydrotoxicarol (**21**)²⁷ and (–)-maackiain (**22**).²⁸

Millexatin A (**1**) exhibited an ion peak at m/z 491.2447 ($[M + H]^+$, calcd 491.2434) in its HRESITOFMS data, which indicated a molecular formula of $C_{30}H_{34}O_6$. Analysis of the NMR spectroscopic data of **1** indicated it is related in structure to isoxantholupon²⁹ which was contained a modified A ring. The major difference between of these two compounds found was that the core structure of isoxantholupon is a flavanone while that in compound **1** is an isoflavanone. Compound **1** showed ^1H NMR resonances for a H-bonded hydroxy proton, an olefinic proton, characteristic of isoflavanones, spin coupled ABC-type aromatic protons, an isoprenyl unit, and a germinal diisoprenyl group (Table 1). The germinal diisoprenyl unit was placed at C-8 due to the HMBC correlations (Figure 1) of H-1'''/H-1'''' (δ_H 2.90 and 2.65) with C-7 (δ_C 194.6), C-8 (δ_C 57.0), and C-9 (δ_C 174.6), whereas the isoprenyl group was placed at C-6 from of the HMBC correlation of H-1'' (δ_H 3.11) with and C-5 (δ_C 162.7), C-6 (δ_C 116.1) and C-7 (δ_C 194.6). It should be noted that griffonianone C³⁰ is the first example of an isoflavone with a modified A ring and was isolated from *M. griffoniana* by Yankep et al. in 2001. However, millexatin A is a rare isoflavone containing three isoprenyl units on a modified A ring.

Millexatin B (**2**) molecular formula $C_{26}H_{26}O_6$ was determined from its HRESITOFMS data ($[M + H]^+$ m/z 435.1804, calcd 435.1808). The NMR spectroscopic data (Table 1) displayed characteristic resonances for a tetraoxygenated isoflavone similar to those of isoauriculatin²² isolated from the leaf extract of *Millettia auriculata*. The major distinction was the replacement of a H-bonded hydroxy group at C-5 in isoauriculatin²² with a methoxy group in **2**. The HMBC correlation between the methoxy protons (δ_H 3.93) and the olefinic proton H-1'' (δ_H 6.78) with C-5 (δ_C 155.0) further verified this deduction.

Millexatin C (**3**) was deduced a molecular formula $C_{28}H_{30}O_7$ from its HRESITOFMS data ($[M + H]^+$ m/z 479.2055, calcd 479.2070). Analysis of the NMR spectroscopic data of **3** (Tables 1 and 2) revealed that it is structurally related to millexatin B (**2**). The major

difference between these two compounds was found on ring B. Compound **2** showed resonances for an ABC-type aromatic proton, while compound **3** exhibited signals for two singlet *para*-aromatic protons at δ_{H} 6.64 and δ_{H} 6.92 and for an additional methoxy group at δ_{H} 3.75. These resonances were assigned to H-3', H-6' and OMe-2', respectively, which were further supported from the following NOESY cross peaks (Figure 1): OMe-5' (δ_{H} 3.85) with H-6' (δ_{H} 6.92) and OMe-2' (δ_{H} 3.75) with H-3' (δ_{H} 6.64).

Millexatin D (**4**) had a molecular formula of $\text{C}_{26}\text{H}_{26}\text{O}_6$ which was deduced from its HRESITOFMS data ($[\text{M} + \text{H}]^+ m/z$ 435.1804, calcd 435.1808). The NMR spectroscopic data of **4** were similar to those of **2**, except for the absence of resonances for an isoprenyloxy unit and H-5' in **4** (Tables 1 and 2). The ^1H NMR spectrum of **4** showed resonances for a 1,1-dimethylallyl group. This unit was attached at C-5', most likely formed via the Claisen rearrangement of the isoprenyl unit of compound **2**. The HMBC correlations also supported this assignment.

Millexatin E (**5**) molecular formula $\text{C}_{25}\text{H}_{22}\text{O}_6$ was deduced from its HRESITOFMS data ($[\text{M} + \text{H}]^+ m/z$ 441.1332, calcd 441.1314). The NMR spectroscopic data (Tables 1 and 2) were similar to those of auriculatin (**10**), having a 1,2,4-trisubstituted B ring. However, compound **5** showed resonances for two pyran rings that were located at C-5/C-6. Correlations in the HMBC spectrum between H-1'' (δ_{H} 6.62) and C-5 (δ_{C} 154.1), C-6 (δ_{C} 107.5), C-7 (δ_{C} 152.7) and between H-1''' (δ_{H} 6.72) and C-7 (δ_{C} 152.7), C-8 (δ_{C} 101.4), C-9 (δ_{C} 152.0) supported the location of these two pyran rings.

Millexatin F (**6**) had a molecular formula of $\text{C}_{25}\text{H}_{24}\text{O}_6$, which was determined from its HRESITOFMS data ($[\text{M} + \text{H}]^+ m/z$ 421.1660, calcd 421.1651). The NMR spectroscopic data (Tables 1 and 2) were very similar to those of millexatin E (**5**), except for the absence of resonances for a pyran ring at C-5/C-6. Compound **6** showed additional resonances for an

isoprenyl unit and a H-bonded hydroxy group (δ_{H} 12.56) at C-5. The HMBC spectrum showed correlations of OH-5 (δ_{H} 12.56) with C-5 (δ_{C} 158.8), C-6 (δ_{C} 113.6), and C-10 (δ_{C} 104.9) and of H-1'' (δ_{H} 3.37) with C-5 (δ_{C} 158.8), C-6 (δ_{C} 113.6) and C-7 (δ_{C} 158.1). Thus, the structure of **6** was elucidated as shown. This structure was found in the SciFinder Scholar database (Chemical Abstracts Service, Columbus, OH, USA), but no references were indicated for this compound, suggesting that it is new.

Putative biogenetic pathways toward the generation of the isoflavones **1-6** are shown in Scheme 1. The intermediate **23.1** could be generated from isoflavones **23** (not isolated from this study) via prenylation at C-8. Further prenylation at C-8 of intermediate **23.1** would give compound **1**. Compound **6** could be also obtained from intermediate **23.1** via cyclization of the isoprenyl group at C-8 onto the OH-7 group. A second cyclization between the isoprenyl group at C-6 and OH-5 group of compound **6** would give compound **5**. A regioselective methylation of the OH-5 followed by cyclization of the isoprenyl group at C-6 with the OH-7 group would give intermediate **23.2**. This upon selective *O*-prenylation at C-4' would produce compound **2**. Claisen rearrangement of **2** could generate compound **4**.³¹ Finally, oxidation of compound **2** at C-5' followed by *O*-dimethylation would then give compound **3**.

Most of the isolated compounds obtained were evaluated for their antibacterial activity against three Gram-positive and two Gram-negative bacteria, as summarized in Table 3. Compounds **1**, **6**, **10**, **11**, and **14** showed potent antibacterial activity against Gram-positive bacteria with MIC values ranging from 2-8 $\mu\text{g/mL}$. Compound **7** showed moderate activity against *S. aureus* with a MIC value of 32 $\mu\text{g/mL}$. All remaining compounds showed either weak (MIC 64–128 $\mu\text{g/mL}$) or no perceptible antibacterial activity against all tested bacterial strains.

EXPERIMENTAL SECTION

General Experimental Procedures. The general information of instruments and materials were the same as previous reports.³²⁻³⁵

Plant Material. The stems of *M. extensa* were obtained in January 2017 from Donmoon Village, Silaphet Sub-district, Pua District, Nan Province, Thailand. Plant authentication was verified by Mr. Martin Van de Bult and a voucher specimen (MFU-NPR0165) was deposited at the Natural Products Research Laboratory of Mae Fah Luang University.

Extraction and Isolation. The chopped and dried stems of *M. extensa* (1.2 kg) were extracted with acetone (6 L) at room temperature (3 days $\times$ 2) and concentrated under reduced pressure to give an acetone extract (194.0 g). The crude extract (76.8 g) was subjected to QCC over silica gel, eluting with 100% hexanes to 100% acetone, to give six fractions (F1-F6). Fractionation of F3 (1.08 g) by Sephadex LH-20 (8:2 MeOH-CH₂Cl₂) gave three subfractions (F3A–F3C). Purification of F3C (124.3 mg) by CC (1:9 EtOAc-hexanes) yielded compounds **8** (1.4 mg), **9** (1.5 mg), **16** (1.7 mg), **17** (22.0 mg), **18** (1.8 mg), and **19** (4.5 mg). Purification of F4 (639.8 mg) by CC (1.5:8.5 EtOAc-hexanes) gave compounds **4** (3.7 mg), **5** (1.8 mg), and **15** (2.7 mg). Fractionation of F5 (13.4 g) by Sephadex LH-20 (9:1 MeOH-CH₂Cl₂) gave four subfractions (F5A-F5D). Separation of F5A (2.7 g) by CC (2:8 acetone-hexanes) yielded compounds **1** (18.0 mg), **2** (2.3 mg), **3** (3.1 mg), and **12** (7.2 mg). Purification of F5C (8.4 g) by CC (2:8 EtOAc-hexanes) yielded compounds **10** (2.2 g), **11** (1.3 g), **13** (25.9 mg), **14** (2.3 mg), **20** (15.2 mg), and **21** (2.5 mg). Compounds **6** (471.0 mg), **7** (7.3 mg), and **22** (41.3 mg) were obtained from F5D (950.1 mg) by CC (0.2:9.8 MeOH-CH₂Cl₂).

Millexatin A (I): Yellow viscous oil; UV (MeOH) λ_{max} (log ϵ) 203 (4.28), 269 (3.93), 349 (3.22) nm; IR (neat) ν_{max} 3373, 2967, 1669, 1621, 1547, 1447 cm⁻¹; ¹H and ¹³C NMR, see Tables 1 and 2; HRESITOFMS m/z 491.2447 [M + H]⁺ (calcd for C₃₀H₃₅O₆, 491.2434).

Millexatin B (2): Yellow viscous oil; UV (MeOH) λ_{max} (log ϵ) 203 (4.15), 225 (3.98), 268 (4.07), 331 (3.38) nm; IR (neat) ν_{max} 3382, 2924, 1607, 1582, 1467 cm^{-1} ; ^1H and ^{13}C NMR, see Tables 1 and 2; HRESITOFMS m/z 435.1804 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{26}\text{H}_{27}\text{O}_6$, 435.1808).

Millexatin C (3): Yellow solid, mp 112-115 $^{\circ}\text{C}$; UV (MeOH) λ_{max} (log ϵ) 272 (4.33), 335 (3.49) nm; IR (neat) ν_{max} 2976, 1639, 1511, 1466 cm^{-1} ; ^1H and ^{13}C NMR, see Tables 1 and 2; HRESITOFMS m/z 479.2055 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{28}\text{H}_{31}\text{O}_7$, 429.2070).

Millexatin D (4): Yellow viscous oil; UV (MeOH) λ_{max} (log ϵ) 291 (4.23), 334 (3.63) nm; IR (neat) ν_{max} 3335, 2973, 1608, 1467 cm^{-1} ; ^1H and ^{13}C NMR, see Tables 1 and 2; HRESITOFMS m/z 435.1804 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{26}\text{H}_{27}\text{O}_6$, 435.1808).

Millexatin E (5): Yellow viscous oil; UV (MeOH) λ_{max} (log ϵ) 296 (4.20), 333 (3.46) nm; IR (neat) ν_{max} 3338, 2976, 1615, 1570, 1366 cm^{-1} ; ^1H and ^{13}C NMR, see Tables 1 and 2; HRESITOFMS m/z 441.1332 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{25}\text{H}_{22}\text{NaO}_6$, 441.1314).

Millexatin F (6): Yellow solid, mp 218-219 $^{\circ}\text{C}$; UV (MeOH) λ_{max} (log ϵ) 202 (4.07) 272 (4.17), 359 (2.91) nm; IR (neat) ν_{max} 3349, 2976, 1639, 1429 cm^{-1} ; ^1H and ^{13}C NMR, see Tables 1 and 2; HRESITOFMS m/z 421.1660 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{25}\text{H}_{25}\text{O}_6$, 421.1651).

Antibacterial Assay. All bacterial strains used in these experiments were obtained from the Microbiological Resources Center, Bangkok, Thailand. The antibacterial assay was determined as recommended by Clinical and Laboratory Standards Institute.^{34,35}

ASSOCIATED CONTENT

The Supporting Information is available free of charge on the ACS Publications website at DOI:

HRESITOFMS, 1D and 2D NMR spectra of new compounds **1–6** (PDF).

AUTHOR INFORMATION

Corresponding Author

*Tel: +66-5391-6238. Fax: +66-5391-6776. E-mail: surat.lap@mfu.ac.th (S. Laphookhieo).

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENT

We are grateful to the Thailand Research Fund and the Mae Fah Luang University for financial support (BRG5980012). We thank Mr. Martin Van de Bult for the plant identification and Ms. Pornphimol Meesakul and Mr. Wuttichai Jaidee for recording the NMR spectra.

REFERENCES

- (1) Ito, C.; Itoigawa, M.; Kumagaya, M.; Okamoto, Y.; Ueda, K.; Nishihara, T.; Kojima, N.; Furukawa, H. *J. Nat. Prod.* **2006**, *69*, 138–141.
- (2) Sritularak, B.; Likhitwitayawuid, K.; Conrad, J.; Vogler, B.; Reeb, S.; Klaiber, I.; Kraus, W. *J. Nat. Prod.* **2002**, *65*, 589–591.
- (3) Deyou, T.; Marco, M.; Heydenreich, M.; Pan, F.; Gruhonjic, A.; Fitzpatrick, P. A.; Koch, A.; Derese, S.; Pelletier, J.; Rissanen, K.; Yenesew, A.; Erdélyi, M. *J. Nat. Prod.* **2017**, *80*, 2060–2066.
- (4) Deyou, T.; Gumula, I.; Pang, F.; Gruhonjic, A.; Mumo, M.; Holleran, J.; Duffy, S.; Fitzpatrick, P. A.; Heydenreich, M.; Landberg, G.; Derese, S.; Avery, V.; Rissanen, K.; Erdélyi, M.; Yenesew, A. *J. Nat. Prod.* **2015**, *78*, 2932–2939.
- (5) Yankep, E.; Njamen, D.; Fotsing, M. T.; Fomum, Z. T.; Mbanya, J.-C.; Giner, R. M.; Recio, M. C.; Manez, S.; Rios, J. L. *J. Nat. Prod.* **2003**, *66*, 1288–1290.
- (6) Kikuchi, H.; Ohtsuki, T.; Koyano, T.; Kowithayakorn, T.; Sakai, T.; Ishibashi, M. *J. Nat. Prod.* **2007**, *70*, 1910–1914.

- (7) Monbrison, F. D.; Maitrejean, M.; Latour, C.; Bugnazet, F.; Peyron, F.; Barron, D.; Picot, S. *Acta Trop.* **2006**, *97*, 102–107.
- (8) Torres-Mendoza, D.; Gonzalez, J.; Ortega-Barria, E.; Heller, M. V.; Capson, T. L.; McPhail, K.; Gerwick, W. H.; Cubilla-Rios, L. *J. Nat. Prod.* **2006**, *69*, 826–828.
- (9) Ntie-Kang, F.; Onguéné, P.A.; Lifongo, L. L.; Ndom, J. C.; Sippl, W.; Mbaze, L. M. *Malar J.* **2014**, *13*, 81.
- (10) Huang, Y.-C.; Hwang, T.-L.; Chang, C.-S.; Yang, Y.-L.; Shen, C.-N.; Liao, W.-Y.; Chen, S.-C.; Liaw, C.-C. *J. Nat. Prod.* **2009**, *72*, 1273–1278.
- (11) Wen, R.; Lv, H.-N.; Jiang, Y.; Tu, P.-F. *Phytochemistry* **2018**, *149*, 56–63.
- (12) Yin, T.; Liang, H.; Wang, B.; Zhao, Y. *Fitoterapia* **2010**, *81*, 274–275.
- (13) Cheng, J.; Zhao, Y.-Y.; Wang, B.; Qiao, L.; Liang, H. *Chem. Pharm. Bull.* **2005**, *53*, 419–421.
- (14) Banzouzi, J. T.; Prost, A.; Rajemiariaho, M.; Ongoka, P. *Int. J. Bot.* **2008**, *4*, 406–420.
- (15) Asres, K.; Mascagni, P.; O'Neill, M. J.; Phillipson, J. D. *Z. Naturforsch.* **1985**, *40c*, 617–620.
- (16) Jain, A. C.; Sharma, B. N. *J. Org. Chem.* **1974**, *39*, 2215–2217.
- (17) Pancharoen, O.; Athipornchai, A.; Panthong, A.; Taylor, W. C. *Chem. Pharm. Bull.* **2008**, *56*, 835–838.
- (18) Huang, Z.; Yang, J.; She, Z.; Lin, Y. *Nat. Prod. Res.* **2012**, *26*, 11–15.
- (19) Shabbir, M.; Zaman, A.; Crombie, L.; Tuck, B.; Whiting, D. A. *J. Chem. Soc. C.* **1968**, 1899–1901.
- (20) Ganapaty, S.; Nair, V.; Rama Devi, D.; Thomas Pannakal, S.; Laatsch, H.; Dittrich, B. *Nat. Prod. Commun.* **2014**, *9*, 937–940.

- (21) Toker, G.; Erdogan Orhan, I. *Gazi Universitesi Eczacilik Fakultesi Dergisi* **1998**, *15*, 29–34.
- (22) MinHaj, N.; Khan, H.; Kapoor, S.K.; Zaman, A. *Tetrahedron* **1975**, *32*, 749–751.
- (23) Asomaning, W. A.; Amoako, C.; Oppong, I. V.; Phillips, W. R.; Addae-Mensah, I.; Osei-Twum, E. Y.; Waibel, R.; Achenbach, H. *Phytochemistry* **1995**, *39*, 1215–1218.
- (24) Smalberger, T. M.; Vleggaar, R.; Weber, J. C. *Tetrahedron* **1975**, *31*, 2297–2301.
- (25) Khalid, S. A.; Waterman, P. G. *Phytochemistry* **1983**, *22*, 1001–1003.
- (26) Shabbir, M.; Zaman, A. *Tetrahedron* **1970**, *26*, 5041–5044.
- (27) Andrei, C. C.; Vieira, P. C.; Fernandes, J. B.; da Silva, M. F. d. G. F.; Rodrigues, F. O. *Phytochemistry* **1997**, *46*, 1081–1085.
- (28) Ding, P.; Qiu, J.-Y.; Ying, G.; Dai, L. *Chin. Herb. Med.* **2014**, *6*, 332–334.
- (29) Dresel, M.; Dunkel, A.; Hofmann, T. *J. Agric. Food Chem.* **2015**, *63*, 3402–3418.
- (30) Wanda, G. J. M. K.; Njamen, D.; Tagatsing, F. M.; Yankep, E.; Vollmer, G. *Pharm. Biol.* **2011**, *49*, 341–347.
- (31) Ren, Y.; Benatrehina, P. A.; Acuña, U. M.; Yuan, C.; Chai, H.-B.; Ninh, T. N.; de Blanco, E. J. C.; Soejarto, D. D.; Kinghorn, A. D. *Planta Med.* **2016**, *82*, 1096–1104.
- (32) Meesakul, P.; Pudhom, K.; Pyne, S. G.; Laphookhieo, S. *RSC Adv.* **2017**, *7*, 17545–17550.
- (33) Sriyatep, T.; Siridechakorn, I.; Maneerat, W.; Pansanit, A.; Ritthiwigrom, T.; Andersen, R.J.; Laphookhieo, S. *J. Nat. Prod.* **2015**, *78*, 265–271.
- (34) Tantapakul, C.; Phakhodee, W.; Ritthiwigrom, T.; Cheenpracha, S.; Prawat, U.; Deachathai, S.; Laphookhieo, S. *J. Nat. Prod.* **2012**, *75*, 1660–1664.
- (35) Tantapakul, C.; Maneerat, C.; Sripisut, T.; Ritthiwigrom, T.; Andersen, R. J.; Cheng, P.; Cheenpracha, S.; Raksat, A.; Laphookhieo, S. *J. Agric. Food Chem.* **2016**, *64*, 8755–8762.

(36) CLSI. Reference Method for Dilution Antimicrobial Susceptibility Test for Bacteria that Grow Aerobically: Approved Standard M07-A9. Clinical and Laboratory Standards Institute: Wayne, PA, 2012.

Figure 1. Selected HMBC correlations ($^1H \rightarrow ^{13}C$) of compounds 1-6.

Scheme 1. Plausible Biosynthetic Pathway for Compounds 1–6.

Table 1. ¹H NMR (600 MHz) Spectroscopic Data of Millexatins A-F (**1–6**) in CDCl₃ (δ in ppm, *J* in Hz)

position	1 ^a	2	3	4	5	6 ^a
2	8.12, s	7.89, s	7.80, s	7.87, s	7.81, s	7.96, s
8	-	6.70, s	6.63, s	6.67, s	-	-
3'	6.59, d (2.0)	6.68, d (2.5)	6.64, s	6.58, s	6.54, d (2.5)	6.56, d (2.5)
5'	6.55, dd (8.5, 2.0)	6.65, dd (8.6, 2.5)	-	-	6.42, dd (8.3, 2.5)	6.48, dd (8.4, 2.5)
6'	7.01, d (8.5)	7.03, d (8.6)	6.92, s	6.93, s	6.94, d (8.3)	7.00, d (8.4)
1''	3.11, d (7.0)	6.78, d (10)	6.76, d (10.0)	6.74, d (10.0)	6.62, d (10.0)	3.37, d (7.3)
2''	5.08, t (7.0)	5.79, d (10)	5.74, d (10.0)	5.76, d (10.0)	5.65, d (10.0)	5.22, br t (7.3)
4''	1.75, s	1.51, s	1.50, s	1.49, s	1.53, s	1.84, s
5''	1.67, s	1.51, s	1.50, s	1.49, s	1.53, s	1.71, s
1'''	2.90, dd (13.7, 7.0); 2.65, dd (13.7, 7.0)	4.54, d (7.0)	4.65, d (6.6)	-	6.72, d (10.0)	6.71, d (10.0)
2'''	4.68, t (7.0)	5.53, t (7.0)	5.55, t (6.6)	6.18, dd (17.6, 10.4)	5.65, d (10.0)	5.62, d (10.0)
3'''	-	-	-	5.29, d (10.4); 5.35, d (17.6)	-	-
4'''	1.55, s	1.82, s	1.81, s	1.43, s	1.50, s	1.49, s
5'''	1.51, s	1.77, s	1.79, s	1.43, s	1.50, s	1.49, s
1''''	2.90, dd (13.7, 7.0); 2.65, dd (13.7, 7.0)	-	-	-	-	-
2''''	4.68, t (7.0)	-	-	-	-	-
4''''	1.55, s	-	-	-	-	-
5''''	1.51, s	-	-	-	-	-
OH-5	12.43, s	-	-	-	-	12.56, s
OMe-5	-	3.93, s	3.91, s	3.90, s	-	-
OH-2'	5.50, br s	9.34, s	-	8.99, s	9.44, br s	8.50, s
OMe-2'	-	-	3.75, s	-	-	-
OH-4'	7.94, s	-	-	5.90, s	5.00, br s	5.09, s
OMe-5'	-	-	3.85, s	-	-	-

^a Recorded on 400 MHz.

Table 2. ^{13}C NMR (150 MHz) Spectroscopic Data of Millexatins A-F (**1–6**) in CDCl_3 (δ in ppm)

position	1 ^a	2	3	4	5	6 ^a
2	154.1	152.0	151.9	152.4	151.8	154.4
3	129.0	125.0	122.0	125.3	124.9	123.0
4	179.6	178.0	174.4	177.8	178.4	182.0
5	162.7	155.0	155.2	155.1	154.1	158.8
6	116.1	113.6	112.8	113.5	107.5	113.6
7	194.6	158.6	157.4	158.5	152.7	158.1
8	57.0	99.9	99.5	99.9	101.4	100.6
9	174.6	157.9	158.2	158.0	152.0	150.3
10	114.4	111.4	112.7	111.6	107.7	104.9
1'	110.8	113.5	111.8	113.1	113.2	112.6
2'	159.1	157.3	151.2	156.1	157.5	157.1
3'	106.7	104.3	100.2	107.7	105.6	106.3
4'	157.6	160.5	148.4	156.4	157.3	158.2
5'	109.1	107.8	143.0	124.6	107.4	108.6
6'	130.9	130.3	115.1	127.2	130.3	130.5
1''	21.0	115.0	115.7	115.3	115.1	21.3
2''	121.5	130.0	130.2	130.7	128.2	121.6
3''	131.9	77.6	77.2	76.9	77.7	131.9
4''	17.9	27.9	27.8	27.9	27.8	17.7
5''	25.8	27.9	27.8	27.9	27.8	25.7
1'''	38.8	64.4	65.6	39.2	114.5	114.6
2'''	116.7	119.0	119.6	147.4	127.2	127.2
3'''	136.0	138.0	137.2	113.1	78.0	78.3
4'''	25.7	25.4	25.4	26.7	27.3	28.0
5'''	17.8	17.7	17.8	26.7	27.3	28.0
1''''	38.8	-	-	-	-	-
2''''	116.7	-	-	-	-	-
3''''	136.0	-	-	-	-	-
4''''	25.7	-	-	-	-	-
5''''	17.8	-	-	-	-	-
OMe-5	-	62.5	62.3	62.6	-	-
OMe-2'	-	-	56.2	-	-	-
OMe-5'	-	-	56.1	-	-	-

^a Recorded on 100 MHz.

Table 3. Antibacterial Activity of Compounds **1**, **6-7**, **10-11**, **13-14**, **17**, **19-20**, and **22**

compound	MIC (µg/mL)				
	Gram-positive bacteria			Gram-negative bacteria	
	<i>S. aureus</i> TISTR 1466	<i>S. epidermidis</i> ATCC 12228	<i>B. subtilis</i> TISTR 008	<i>S.typhimurium</i> TISTR781	<i>Ps. aeruginosa</i> TISTR 292
1	2	2	2	128	128
6	2	2	2	128	128
7	32	64	64	128	128
10	2	2	2	128	128
11	2	4	2	128	128
13	inactive	inactive	inactive	128	128
14	4	4	8	128	128
17	inactive	inactive	inactive	128	128
19	inactive	inactive	inactive	128	128
20	inactive	inactive	inactive	128	128
22	inactive	inactive	inactive	128	128
vancomycin	0.25	0.25	0.25	Not tested	Not tested
gentamycin	Not tested	Not tested	Not tested	0.25	2

^aInactive at >128 µg/mL.

Table of Content

Antibacterial Prenylated Isoflavonoids from the Stems of *Millettia extensa*

Achara Raksat, Wisanu Maneerat, Raymond J .Andersen, Stephen G. Pyne and Surat Laphookhieo