1-1-2014

Carbazole alkaloids and coumarins from the roots of Clausena guillauminii

Chiramet Auranwiwat
Chiang Mai University

Surat Laphookhieo
Mae Fah Luang University

Kongkiat Trisuwan
Chiang Mai University

Stephen G. Pyne
University of Wollongong, spyne@uow.edu.au

Thunwadee Ritthiwigrom
Chiang Mai University, tr379@uow.edu.au

Carbazole alkaloids and coumarins from the roots of Clausena guillauminii

Abstract

Two novel carbazole alkaloids, guillauminines A and B (1 and 2), and sixteen known compounds were isolated and identified from the acetone extract of Clausena guillauminii roots. Their structures were elucidated by spectroscopic methods. The cytotoxic, antimalarial and antimycobacterial activities of the isolated compounds were evaluated.

Keywords

Clausena guillauminii, Carbazole alkaloid, Coumarin, Cytotoxic activity, Antimalarial activity, Antimycobacterial activity, CMMB

Disciplines

Medicine and Health Sciences | Social and Behavioral Sciences

Publication Details

Auranwiwat, C., Laphookhieo, S., Trisuwan, K., Pyne, S. G. & Ritthiwigrom, T. (2014). Carbazole alkaloids and coumarins from the roots of Clausena guillauminii. Phytochemistry Letters, 9 113-116.

Carbazole alkaloids and coumarins from the roots of *Clausena guillauminii*

Chiramet Auranwiwat[a], Surat Laphookhieo[b], Kongkiat Trisuwan[a], Stephen G. Pyne[c], Thunwadee Ritthiwigrom[a,*]

[a] *Department of Chemistry, Faculty of Science, Chiang Mai University, Sutep, Muang, Chiang Mai 50200, Thailand*

[b] *Natural Products Research Laboratory, School of Science, Mae Fah Luang University, Tasud, Muang, Chiang Rai 57100, Thailand*

[c] *School of Chemistry, University of Wollongong, Wollongong, New South Wales 2522, Australia*

* Corresponding author. Tel: +66 5394 3341 ext 151; Fax: +66 5389 2277.

E-mail address: thunwadee.r@cmu.ac.th, othunwadee@gmail.com (T. Ritthiwigrom)

Abstract: Two novel carbazole alkaloids, guillauminines A and B (**1** and **2**), and sixteen known compounds were isolated and identified from the acetone extract of *Clausena guillauminii* roots. Their structures were elucidated by spectroscopic methods. The cytotoxic, antimalarial and antimycobacterial activities of the isolated compounds were evaluated.

Keywords: *Clausena guillauminii*, Carbazole alkaloid, Coumarin, Cytotoxic activity, Antimalarial activity, Antimycobacterial activity.

1. Introduction

Clausena is an abundant source of secondary metabolites, especially carbazole alkaloids (Maneerat et al., 2011, 2012, 2013; Songsiang et al., 2011, 2012; Thongthoom et al., 2010), coumarins (Maneerat et al., 2012; Songsiang et al., 2011, 2012; Thongthoom et al., 2010) and amide derivitives (Riemer et al., 1997; Wang et al., 2013). Many of the isolated compounds have a wide range of pharmacological activities including anticancer (Maneerat et al., 2012, 2013; Songsiang et al., 2011; Thongthoom et al., 2010), anti-HIV (Kongkathip et al., 2005, 2010; Krahl et al., 2006), anti-malarial (Maneerat et al., 2011; Thongthoom et al., 2010) and anti-TB (Maneerat et al., 2011; Thongthoom et al., 2010). In this paper, we investigated the phytochemicals from the roots of *Clausena guillauminii* (Rutaceae family), which led to the isolation and characterization of two new carbazole alkaloids (**1** and **2**) along with sixteen known compounds (**3**-**18**). The structures of the isolated compound were elucidated using spectroscopic methods especially 1D and 2D NMR spectroscopy. The structures of the known compounds were determined and confirmed by comparison of their ^{1}H and/or ^{13}C spectroscopic data with those reported in the literature. The cytotoxic, antimalarial and antimycobacterial activities of the isolated compounds were examined.

2. Results and discussion

Investigation of the acetone extract from the air-dired roots *C. guillauminii* led to the isolation of two new carbazole alkaloids, guillauminines A and B (**1** and **2**); fourteen known carbazole alkaloids, heptahylline (**3**) (Songsiang et al., 2012), 7-methoxyheptaphylline (**4**) (Songsiang et al., 2012), 3-formyl-7-hydroxy-9*H*-carbazole (**5**) (Ito et al., 1992; Krahl et al., 2006), fluroclausine A (**6**) (Wu et al., 1997), mukonal (**7**) (Thongthoom et al., 2010), 7-methoxymukomal (**8**) (Songsiang et al., 2012), clauszoline K (**9**) (Thongthoom et al., 2010), 7-

methoxymurrayacine (**10**) (Songsiang et al., 2012), clauraila D (**11**) (Songsiang et al., 2011), heptazoline (**12**) (Songsiang et al., 2012), glycosinine (**13**) (Thongthoom et al., 2010), 7-hydroxyheptaphylline (**14**) (Songsiang et al., 2012), 3-formyl-2,7-dimethoxycarbazole (**15**) (Ruangrungsi et al., 1990) and clauszoline C (**16**) (Krahl et al., 2013); and two known coumarins, osthol (**17**) (Thongthoom et al., 2010) and xanthoxyletin (**18**) (Songsiang et al., 2012) (Fig. 1).

Guillauminine A (**1**) was obtained as a yellow solid with $[\alpha]_D^{24}$ -0.06° (*c* 0.04, MeOH) and mp 234.0-235.0 °C. Its molecular formula, $C_{19}H_{19}NO_5$, was deduced from HRESI-TOFMS. The IR spectrum indicated the presence of a hydroxyl, amine and aldehyde groups at 3366, 3212 and 1653 cm^{-1}, respectively (Wu et al., 1997 and Thongthoom et al., 2010). The UV spectrum revealed the maxima absorption bands at λ_{max} 241, 279, 301 and 347 nm indicating that **1** had a carbazole alkaloid chromophore (Maneerat et al., 2011, 2012, 2013; Songsiang et al., 2011, 2012; Thongthoom et al., 2010). The ^{1}H NMR spectrum (Table 1) displayed characteristic signals for an aldehyde proton (δ 10.42, s, 1H), an amino proton (δ 10.32, br s, 1H), three aromatic protons of a 1,2,4-trisubstituted benzene ring [δ 7.96 (d, J = 8.5 Hz, 1H, H-5), 7.14 (d, J = 2.1 Hz, 1H, H-8) and 6.83 (dd, J = 8.5, 2.1 Hz,1H, H-6)], a singlet aromatic proton (δ 8.30, s, 1H, H-4), two oxymethine protons [δ 4.96 (d, J = 7.9 Hz, 1H, H-4') and δ 3.82 (d, J = 7.9 Hz, 1H, H-3')], a methoxy group (δ 3.84, s, 3H) and two methyl groups (δ 1.56 and 1.32, each s, 3H). Compound **1** showed nine quaternary (δ 160.0, 154.2, 144.9, 143.3, 119.0, 118.7, 117.9, 107.6 and 80.9), seven methine (δ 188.9, 121.4, 119.4, 109.5, 96.7, 76.8 and 69.4) and three methyl (δ 55.8, 27.0 and 19.5) carbons in the ^{13}C NMR and DEPT 135 spectra (Table 1). The aromatic protons at δ 7.96, 7.14 and 6.83 were assigned to be H-5, H-8 and H-6, respectively, due to their multiplicity and HMBC correlations (Table 1), H-5/C-4a (δ 118.7), C-4b (δ 119.0), C-7 (δ 160.0) and C-8a (δ 143.3), H-8/ C-4b, C-6 (δ 109.5), C-7 and C-8a and H-6/C-4b, C-7 and C-8 (δ 96.7).

The methoxy group resonating at δ 3.84 was located at C-7 (δ 160.0) on the basis of the HMBC cross peak between the methoxy protons with C-7 and the chemical shifts of C-6 and C-8. The aldehyde proton, (δ 10.42), showed HMBC cross peaks with C-3 (δ 117.9) and C-4 (δ 119.4), suggesting the location of the formyl group at C-3. The singlet aromatic proton at δ 8.30 was assigned to H-4 according to its HMQC cross peak with C-4 (δ 119.4) as well as the HMBC correlations with C-2 (δ 154.2), C-3 (δ 117.9), C-9a (δ 144.9) and the aldehyde carbon (δ 188.9). In the ^{1}H-^{1}H COSY spectrum, the oxymethine proton, H-4' (δ 4.96) coupled with the other oxymethine proton, H-3' (δ 3.82), which correlated with C-2' (δ 80.9), C-4' (δ 69.4), C-5' (δ 27.0) and C-6' (δ 19.5) in the HMBC spectrum. These data together with the chemical shift of C-2 and the HMBC cross peaks H-4'/C-1 (δ 107.6), C-2 (δ 154.2) and C-9a (δ 144.9) indicated that a dimethylchromane ring with dihydroxy groups at C-3' and C-4' was fused at C-1 and C-2 of the carbazole nucleus with an ether linkage at C-2. The oxymethine protons, H-3' and H-4', were located in pseudoaxial positions on the basis of the large coupling constant of 7.9 Hz between H-3' and H-4' (Wu et al., 1997).

Guillauminine B (**2**) was obtained as a brown solid with $[\alpha]_D^{24}$ -1.15° (*c* 0.05, MeOH) and mp 145.0-147.0 °C. Its molecular formula, $C_{19}H_{19}NO_4$, was deduced from HRESI-TOFMS. The IR, UV, ^{1}H and ^{13}C NMR spectral data were similar to those of compound **1** and the spectroscopic data compound **2** was almost identical to that of synthetic **2** reported ealier (Thongthoom et al., 2011).

Guillauminines A and B (**1** and **2**) have similar structures to clausines W and T (Wu et al., 1997), respectively, with replacement the hydroxyl group at C-7 in these known alkaloids with a methoxyl group in **1** and **2**. The absolute configuration in compounds **1** and **2** should be

racemic, which found to have low specific rotation, $[\alpha]_D^{24}$ -0.06° (*c* 0.04, MeOH) and $[\alpha]_D^{24}$ -1.15° (*c* 0.05, MeOH), respectively.

In a previous report, two known carbazole alkaloids (**3** and **4**) and three coumarins (poncitrin, **17** and **18**) were isolated from the roots of *C. guillauminii* (Nakamura et al., 2009). Poncitrin and **17** inhibited iNOS protein expression while **18** inhibited TNF-α, and COX-2 expression in mouse macrophage RAW 264.7 (Nakamura et al., 2009). In contrast, our study revealed the isolation of sixteen carbazole alkaloids (**1-16**) from this same plant. All isolated compounds were evaluated for their cytotoxicity against two human cancer lines, NCI-H187 (small cell lung cancer) and KB (oral cavity cancer), Vero cell lines (African green monkey kidney, normal cells) (Table 2) and their antimalaria (against *Plasmodium falciparum*) and antimycobacterial (against *Mycobacterium tuberculosis*) activities.

Compounds **2-3**, **5**, **9**, **10**, **15** and **18** were inactive to all tested cell lines (NCI-H187, KB, Vero cell, *P. falciparum* and *M. tuberculosis*). Compounds **6** and **14** displayed the highest cytotoxic activities against the NCI-H187 cell line with IC_{50} values of 7.44 and 9.51 µg/mL, respectively. Compound **6** showed stronger activity against KB cell line with an IC_{50} value of 1.35 µg/mL. In addition compound **7** was non cytotoxic to the Vero cell line. Furthermore, compounds **7**, **8** and **11** exhibited antimalaria activity with IC_{50} values of 4.03, 3.46 and 3.41 µg/mL, respectively, as well as compounds **6** and **14** displayed weak antimycobacterial with the same IC_{50} value of 25 µg/mL.

3. Experimental

3.1 General

Optical rotations were measured in MeOH at the sodium D-line on a Bellingham & Stanley ADP220 polarimeter. UV-vis absorption spectra were determined in MeOH with a Perkin-Elmer UV-vis spectrophotometer. The infrared (IR) spectra were recorded on neat samples using a Perkin-Elmer FTS FT-IR spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a 400 MHz Bruker FTNMR Ultra Shield spectrometer. Chemical shifts are expressed in δ (ppm) referring to the tetramethylsilane (TMS) peak. Mass data were obtained on a MicroTOF, Bruker Daltonics mass spectrometer. Melting point were measured on a SANYO Gallenkamp melting point apparatus. Thin-layer chromatography (TLC) was performed on silica gel 60 GF_{254} (Merck). Column chromatography (CC) was carried out on Sephadex LH-20, and silica gel (Merck) type 100 (63-200 μm) and type (5-40 μm for QCC). All solvents for extraction and chromatography were routinely distilled prior to use.

3.2 Plant material

The roots of *C. guillauminii* were collected at Nong Khai Province, Thailand in March 2011. The plant was identified by Mr. James Maxwell and Dr. Monthon Norsaengsri. Voucher specimen numbers QBG 45329 and QBG 45330 were deposited at the herbarium collection of Queen Sirikit Botanic Garden, Mae Rim, Chiang Mai, Thailand.

3.3 Extraction and isolation

The dried roots of *C. guillauminii* (9.7 kg) were extracted with acetone over a period of 3 days at room temperature. Removal of the solvent under reduced pressure provided the acetone extract (385.3 g) as a dark brown gum. The crude extract was separated by QCC over silica gel and eluted with a gradient of hexanes-CH_2Cl_2-acetone to give ten fractions (A–J). Fraction B (1.5 g) was washed with hexanes to give compound **3** (326.0 mg) as a yellow solid. Fraction D (7.6 g) was further purified by CC over silica gel with 15% EtOAc-hexanes to afford four subfractions (D1-D4). Compounds **4** (1.0 g), **17** (316.0 mg) and **18** (77.4 mg) were obtained from subfraction

D2 (2.5 g) after purification by CC over Sephadex LH-20 with MeOH/CH_2Cl_2 (4:1). Fraction F (4.5 g) was isolated by QCC with a gradient of hexanes-acetone giving four subfractions (F1-F4). Subfraction F2 (139.8 mg) was fractionated by CC over silica gel with CH_2Cl_2/hexanes (4:1) to afford five subfractions (F2A-F2E). Compound **5** (2.3 mg) was contained in the second subfraction. Compound **2** (3.0 mg) was purified from the subfraction F2C after CC over silica gel eluted with EtOAc/CH_2Cl_2 (1:4). Subfraction F3 (551.2 mg) was separated by QCC with EtOAc/CH_2Cl_2 (1:9) giving five subfractions (F3A-F3E). The second subfraction (119.0 mg) was further purified by CC over silica gel with EtOAc/CH_2Cl_2 (1:4), followed by Sephadex LH-20 CC with 100% MeOH to provide compounds **2** (7.5 mg) and **6** (8.2 mg). Compound **1** (14.0 mg) was purified from the fourth subfraction (61.8 mg) after separation using Sephadex LH-20 CC with 100% MeOH. Fraction G (6.7 g) was isolated by CC over silica gel with EtOAc/hexanes (1:4) to give five subfractions (G1-G5). Compound **7** (20.5 mg) was purified from subfraction G2 (183.3 mg) after separation by Sephadex LH-20 CC with 100% MeOH. Subfraction G4 (1.8 g) was fractionated by CC over silica gel with EtOAc/hexanes (1:4) giving five subfractions (G4A-G4E). Compound **8** (56.3 mg) was obtained from the last subfraction. Compounds **7** (4.0 mg) and **9** (6.3 mg) were obtained from the third subfraction (200.0 mg) after purification by CC over silica gel with EtOAc/hexanes (3:7). Fraction I (35.8 g) was further purified by QCC with a gradient of hexanes-CH_2Cl_2-EtOAc to give six subfractions (I1-I6). Purification of subfraction I2 (273.0 mg) by CC over silica gel with CH_2Cl_2/hexanes (4:1) gave four subfractions (I2A-I2D). Compound **13** (66.0 mg) was contained in the first subfraction. The second subfraction (51.6 mg) was further purified by Sephadex LH-20 CC with 100% MeOH to give compound **10** (2.0 mg). Compounds **11** (2.0 mg) and **12** (8.0 mg) were achieved from subfraction I2B by CC over silica gel with EtOAc/hexanes (1.5:8.5). Subfraction I4 (10.0 g) was isolated by QCC with a gradient

of hexanes-CH_2Cl_2 to give six subfractions (I4A-I4F). Compound **14** (100.0 mg) was purified from the first subfraction after isolation using CC over silica gel with acetone/CH_2Cl_2 (1:49). Subfractions I4C (2.5 g) was further purified by CC over silica gel with acetone/hexanes (1:49) to provide five subfractions (I4CA-I4CE). Compound **16** (9.3 mg) was obtained in the first subfraction. The second subfraction (119.0 mg) was further purified by Sephadex LH-20 CC with 100% MeOH to give compound **15** (43.0 mg).

3.3.1 Guillauminine A (**1**)

Yellow solid; mp. 234.0-235.0 °C; $[\alpha]_D^{25}$ -0.06° (c 0.05, MeOH); UV (MeOH) λ_{max} nm (log ε) 347 (3.82), 301 (4.19), 279 (4.14), 241 (4.29); IR (KBr) ν_{max} cm^{-1} 3366, 3212, 1653; ^{1}H-NMR (400 MHz, Acetone-d_6) and ^{13}C-NMR (100 MHz, Acetone-d_6) see Table 1; HRMS *m/z*: 364.1147 $[M+Na]^+$ (calcd. for $C_{19}H_{19}NO_5$+Na, 364.1161).

3.3.2 Guillauminine B (**2**)

Brown solid; mp. 145.0-147.0 °C; $[\alpha]_D^{24}$ -1.15° (c 0.04, MeOH); UV (MeOH) λ_{max} nm (log ε) 345 (3.86), 301 (4.17), 279 (4.17), 241 (4.36); IR (KBr) ν_{max} cm^{-1} 3360 , 3179, 1653; ^{1}H-NMR (400 MHz, Acetone-d_6) and ^{13}C-NMR (100 MHz, Acetone-d_6) see Table 1; HRMS *m/z*: 348.1216 $[M+Na]^+$ (calcd. for $C_{19}H_{19}NO_4$+Na, 348.1212).

3.4 Bioactivity assays

The activities against KB and NCI-H187 cell lines were evaluated using the resazurin microplate assay (O'Brien et al., 2000). The activity assay against African green monkey kidney fibroblast (Vero) cells was performed in triplicate employing the method described by Hunt et al., 1999. The in vitro anti-malaria activity against *Plasmodium falciparum* (K1, multidrug-

resistant strain) was assessed using the microculture radioisotope technique described by Desjardins et al., 1979. The standard compounds were dihydroartemisinin and mefloquine. The antimycobacterial activity was performed against *Mycobacterium tuberculosis* H_{37}Ra strain using the green fluorescent protein microplate assay (GFPMA) by Collins and Franzblau, 1997. The standard compounds, rifampicin, streptomycin, isoniazid, ofloxacin and ethambutol, were used as the reference substances.

Acknowledgments

This work was supported by the Bioresources Research Network, National Center for Genetic Engineering and Biotechnology for research grant (Grant number BRN 001 G-56). C. Auranwiwat thanks the Science Achievement Scholarship of Thailand (SAST) for a scholarship. The Department of Chemistry, Faculty of Science, Chiang Mai University is gratefully acknowledged for partial support.

References

Collins, L.A., Franzblau, S.G., 1997. Microplate Alamar blue assay versus BACTEC 460 system for high-throughput screening of compounds against *Mycobacterium tuberculosis* and *Mycobacterium avium.* Antimicrob. Agents Chemother. 41, 1004-1009.

Desjardins, R.E., Canfield, C.J., Chulay, D., 1979. Quantitative assessment of antimalarial activity in vitro by a semiautomated microdilution technique. Antimicrob. Agents Chemother. 16, 710-718.

Hunt, L., Jordan, M., De Jesus, M., Wurm, F. M., 1999. GFP-expressing mammalian cells for fast, sensitive, noninvasive cell growth assessment in a kinetic mode. Biotechnol. Bioeng. 65, 201–205.

Ito, C., Okahana, N., Wu, T.-S., Wang, M.-L., Lai, J.-S., Kuoh, C.-S., Furukawa, H., 1992. New carbazole alkaloids *from Murraya euchrestifolia*. Chem. Pharm. Bull. 40, 230-232.

Kongkathip, B., Kongkathip, N., Sunthitikawinsakul, A., Napaswat, C., Yoosook, C., 2005, Anti-HIV-1 constituents from *Clausena excavata*: Part II. Carbazoles and a pyranocoumarin. Phytother. Res. 19, 728-731.

Kongkathip, B., Sutthiprabha, S., Yoosook, C., Mongkolsook, Y., Kongkathip, N. J., 2010. Determination of a pyranocoumarin and three carbazole compounds in *Clausena excavata* by RP-HPLC. Chromatogr. Sci. 48, 445-449.

Krahl, P.M., Jager, A., Krause, T., Knolker, J.-H., 2006. First total synthesis of the 7-oxygenated carbazole alkaloids clauszoline-K, 3-formyl-7-hydroxycarbazole, clausine M, clausine N and the anti-HIV active siamenol using a highly efficient palladium-catalyzed approach. Org. Biomol. Chem. 4, 3215-3219.

Krahl, P.K., Kataeva, O., Schmidt, W.A., Knolker, H.-J., 2013. Iron-mediated total synthesis of 2,7-dioxygenated carbazole alkaloids. Eur. J. Org. Chem. 1, 59-64.

Maneerat, W., Phakhodee, W., Cheenpracha, S., Ritthiwigrom, T., Deachathai, S., Laphookhieo, S., 2013. Clausenawallines G-K, carbazole alkaloids from *Clausena wallichii* twigs. Phytochemistry 88, 74-78.

Maneerat, W., Ritthiwigrom, T., Cheenpracha, S., Laphookieo, S., 2012. Carbazole alkaloids and coumarins from *Clausena lansium* roots. Phytochem. Lett. 5, 26-28.

Maneerat, W., Ritthiwigrom, T., Cheenpracha, S., Prawat, U., Laphookhieo, S., 2011. Clausenawallines and B, two new dimeric carbazole alkaloids from the roots of *Clausena wallichii*. Tetrahedron Lett. 52, 3303-3305.

Nakamura, T., Kodama, N., Arai, Y., Kumamoto, T., Higuchi, Y., Chaichantipyuth, C., Ishikawa, T., Ueno, K., Yano, S., 2009. Inhibitory effect of oxycoumarins isolated from the Thai medicinal plant *Clausena guillauminii* on the inflammation mediators, iNOS, TNF-α, and COX-2 expression in mouse macrophage RAW 264.7. J. Nat. Med. 63, 21-27.

O'Brien, J., Wilson, I., Orton, T., Pognan, F., 2000. Investigation of the Alamar Blue (resazurin) fluorescent dye for the assessment of mammalian cell cytotoxicity. Eur. J. Biochem. 267, 5421–5426.

Riemer, B., Hofer, O., Greger, H., 1997. Tryptamine derived amides from *Clausena indica*. Phytochemistry 45, 337-341.

Ruangrungsi, N., Ariyaprayoon, J., 1990. Studies on Thai medicinal plants. Part XI. Three new carbazole alkaloids isolated from *Murraya siamensis*. J. Nat. Prod. 53, 946-952.

Songsiang, U., Thongthoom, T., Boonyarat, C., Yenjai, C., 2011. Claurailas A-D, cytotoxic carbazole alkaloids from the roots of *Clausena harmandiana*. J. Nat. Prod. 74, 208-212.

Songsiang, U., Thongthoom, T., Zeekpudsa, P., Kukongviriyapan, V., Boonyarat, C., Wangboonskul, J., Yenjai, C. 2012. Antioxidant activity and cytotoxicity against cholangiocarcinoma of carbazoles and coumarins from *Clausena harmandiana.* ScienceAsia. 38, 75-81.

Thongthoom, T., Promsuwan P., Yenjai, C., 2011. Synthesis and cytotoxic activity of the heptaphylline and 7-methoxyheptaphylline series. Eur. J. Med. Chem, 46, 3755-3761**.**

Thongthoom, T., Songsiang, U., Phaosiri, C., Yenjai, C., 2010. Biological activity of chemical constituents from *Clausena harmandiana*. Arch. Pharm. Res. 33, 675-680.

Wang, G.-C.**,** Li, W.**,** Wang, Y.**,** Zhang, X.-Q.**,** Li, Y.-L.**,** Ye, W.-C.**,** 2013. A new amide and a new monoterpene from the seeds of *Clausena lansium*. Nat. Prod. Res. 27, 558-562.

Wu, T.-S., Huang, S.-C., Wu, P.-L., 1997. Pyrano- and furocarbazole alkaloids from the root bark of *Clausena excavate*. Heterocycles 45, 969-973.

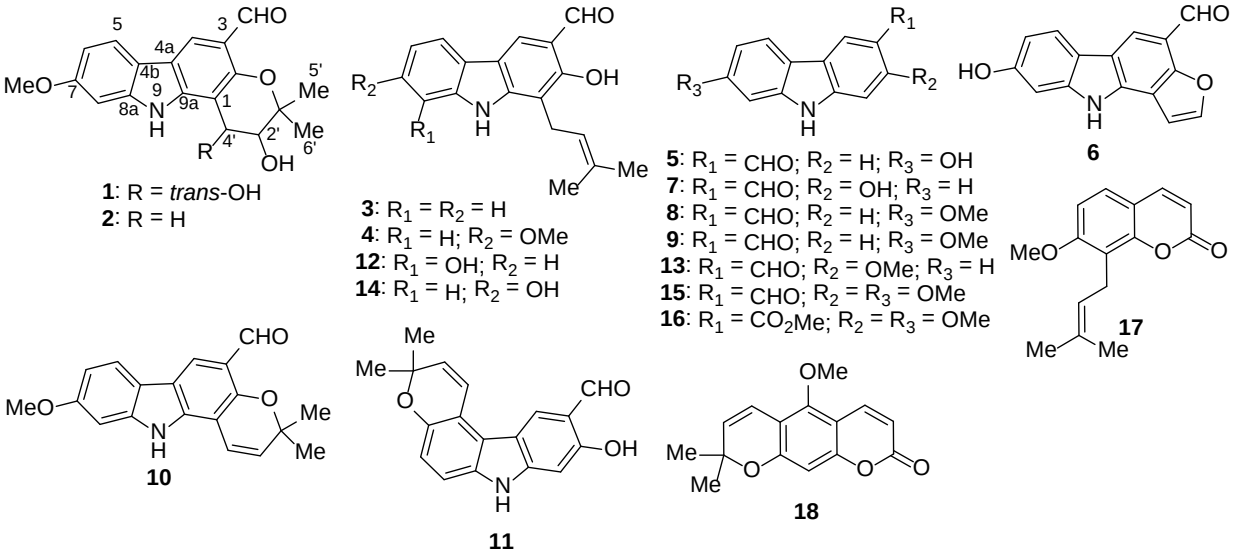

Fig. 1. Structures of the isolated compounds **1**-**18** from *C. guillauminii* roots.

Table 1 NMR spectral data of guillauminines A and B (**1** and **2**) in acetone-d_6 at 400 MHz

Position	**1**				**2**			
	δ_H (mult., *J* in Hz)	δ_C		HMBC	δ_H (mult., *J* in Hz)	δ_C		HMBC
1	–	107.6		–	–	103.7		–
2	–	154.2		–	–	154.6		–
3	–	117.9		–	–	120.6		–
4	8.30 (s)	119.4	CH	C-2, C-3, C-9a, CHO	8.24 (s)	117.9	CH	C-2, C-9a, CHO
4a	–	118.7		–	–	120.9		–
4b	–	119.0		–	–	119.3		–
5	7.96 (d, 8.5)	121.4	CH	C-4a, C-4b, C-7, C-8a	7.97 (d, 8.5)	121.4	CH	C-4a, C-7, C-8a
6	6.83 (dd, 8.5, 2.1)	109.5	CH	C-4b, C-7, C-8	6.84 (dd, 8.5, 2.1)	109.3	CH	C-4b, C-8
7	–	160.0		–	–	159.9		–
8	7.14 (d, 2.1)	96.7	CH	C-4b, C-6, C-7, C-8a	7.00 (d, 2.1)	96.4	CH	C-4b, C-6, C-7, C-8a
8a	–	143.3		–	–	143.2		–
9a	–	144.9		–	–	145.1		–
2'	–	80.9		–	–	79.0		–
3'	3.82 (d, 7.9)	76.8	CH	C-2', C-4', C-5', C-6'	4.01 (m)	69.1	CH	C-2', C-5', C-6'
4'	4.96 (d, 7.9)	69.4	CH	C-1, C-2, C-9a, C-3'	a 3.23 (dd, 16.5, 5.7)	28.1	CH_2	C-1, C-2, C-9a
	–	–		–	b 2.90 (dd, 16.5, 7.2)			C-2', C-3'
5'	1.56 (s)	27.0	CH_3	C-2', C-3', C-6'	1.48 (s)	20.7	CH_3	C-2', C-3', C-6'
6'	1.32 (s)	19.5	CH_3	C-2', C-3', C-5'	1.38 (s)	25.9	CH_3	C-2', C-3', C-5'
CHO	10.42 (s)	188.9	CH	C-3, C-4	10.46 (s)	189.9	CH	C-3, C-4
7-OMe	3.84 (s)	55.8	CH_3	C-7	3.85 (s)	55.8	CH_3	C-7
3'-OH	–	–		–	4.53 (d, 5.4)	–		–
9-NH	10.32 (br s)	–		–	10.44 (br s)	–		–