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Zanthoamides G-I: Three New Alkamides from *Zanthoxylum zanthoxyloides*

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ABSTRACT

Three new alkamides, zanthoamides G-I (1-3), together with ten known compounds, araliopsine, skimmianine, \(N\)-methylplatydesminium cation, isoplatydesmine, myrtopsine, atanine, \(N\)-methylatanine, sesamin, hesperetin and hesperidin, were isolated from the fruits of *Zanthoxylum zanthoxyloides*. Their structures were elucidated by spectroscopic means. All isolated compounds were assessed for their cytotoxicity against A549, MCF7, PC3 and PNT2 cell lines. Among the alkamides, only zanthoamide G (1) showed low level of cytotoxicity against MCF7 cells.

*Keywords:*
*Zanthoxylum zanthoxyloides*; Rutaceae; Cytotoxicity; Alkamides; Zanthoamides
1. Introduction

The genus *Zanthoxylum* L. (family Rutaceae) consists of ca. 250 species of trees and shrubs, distributed throughout tropical and temperate regions of the world (Global Biodiversity Information Facility, 2017). In Africa, this genus is represented by 35 species, many of which are used as spices, edible fruits, medicinal plants and wood for construction (Chase et al., 1999; Seidemann et al., 2005; Matu, 2011). *Zanthoxylum zanthoxyloides* (Lam.) Zepern. & Timler (synonyms - *Fagara zanthoxyloides* Lam. and *Zanthoxylum senegalense* DC), one of the African *Zanthoxylum* species, is a small tree (6-8 m), which is widely cited in African traditional medicine. Different parts of the plant are traditionally used in the forms of infusion and decoction to treat diseases such as malaria, fever, sickle cell anemia, tuberculosis, paralysis, elephantiasis, toothache, venereal diseases, dysmenorrhea, abdominal pain, cough, tuberculosis, urinary disorders, cancers and arthritis (Odebeyi and Sofowora, 1979; Amvam et al., 1998). In Cameroon, a decoction from *Z. zanthoxyloides* fruits is used for the treatment of cancers, sickle cell anemia and as a spice to cook special potages used as a purgative (Adjanohoun et al., 1986; Tchiegang and Mbougueng, 2005). Previous phytochemical studies on the fruits revealed the presence of quinic acid derivatives and acridone alkaloids with aromatase properties (Ouattara et al., 2009; Wouatsa et al., 2013). Antifungal and antioxidant isobutylamides and cytotoxic benzophenanthridine alkaloids were reported from the roots (Sofowora et al., 1975; Chaib et al., 2003; Adesina, 2005; Queiros et al., 2006). Other previously isolated compounds from the plant include aporphine and quinoline alkaloids, coumarins, lignans and various phenolic compounds (Adesina, 2005). In this paper, in continuation of our studies on Cameroonian medicinal plants (Wansi et al., 2016; Guetchueng et al., 2017, 2018; Tahsin et al., 2017), we now report on the isolation and identification of three new alkamides, zanthoamines G-I (1-3), together with ten known compounds (4-13) from the fruits of *Z. zanthoxyloides*, collected from Cameroon, and assessment of their cytotoxicity against three human cancer cell lines, adenocarcinomic alveolar basal epithelial (A549), breast adenocarcinoma (MCF7) and prostate cancer (PC3) and the normal human prostate epithelium (PNT2) cell lines.
2. Results and discussion

Reversed-phase preparative HPLC purification of the methanolic extract of the fruits of Z. zanthoxyloides afforded three new alkamides, named zanthoamides G-I (1-3), along with ten known compounds including: eight quinoline alkaloids, araliopsine (4), skimmianine (5), N-methylplatydesminium cation (6), isoplatydesmine (7), myrtopsine (8), atanine (9) and N-methylatanine (10), a lignan, sesamin (11) and two flavanones, hesperetin (12) and hesperidin (13) (Fig. 1). Whilst the new compounds were identified by extensive 1D and 2D NMR and HRMS analyses, the known compounds were identified by comparison of their spectroscopic data with respective literature data (Brown et al., 1980; Ngadjui et al., 1988; Liu et al., 2000; El-Shafae, 2002; Albarici et al., 2010; Haque et al., 2013).

Compounds 1-3 were obtained as white viscous liquids. The molecular formula of compound 1 was determined as C\textsubscript{18}H\textsubscript{29}NO\textsubscript{4} by HRESIMS from the pseudomolecular ion at m/z 346.1984, calculated 346.1989 for its sodium adduct [M + Na]\textsuperscript{+} (C\textsubscript{18}H\textsubscript{28}NO\textsubscript{4}Na). The \textsuperscript{1}H NMR spectrum of 1 (Table 1) exhibited 14 signals corresponding to 24 protons including the signals of the proton of a secondary amine at 8.56 (1H, br s), six olefinic protons at δ7.12 (1H, dd, J = 10.4, 15.1), 6.60 (1H, dd, J = 11.1, 15.1), 6.27 (1H, dd, J = 10.8, 15.1), δ5.99 (1H, d, J = 15.0), 5.70 (1H, dd, J = 6.4, 15.0) and 5.44 (1H, m, J = 7.4), signals at δ1.18 (6H, s) and 1.11 (3H, d, J = 6.3) for three methyls, two oxygenated methines at δ3.58 (1H, m, J = 6.3) and 3.91 (1H, dd, J = 6.4, 12.9) and three methylene signals including a broad singlet at δ3.27 and two doublets at δ2.29 (J = 7.0, 13.2) and 2.35 (J = 7.3, 14.8). Overlapping signals occurring between δ6.0-6.15 ppm resulting from two olefinic protons resonances were also observed. The \textsuperscript{13}C NMR spectrum (Table 1) revealed signals for 18 carbons attributable to three methyls (δ27.2 x 2, δ18.8), three methylenes with one appearing relatively downfield at δ51.1, ten methines including two oxymethines at δ77.8 and 71.7, eight olefinic methines at δ143.0, 142.3, 134.0, 131.7, 130.2, 129.9, 128.2 and 123.2, and two quaternary carbons including an amide at δ169.4 and an oxygenated quaternary carbon at δ71.6. The assignment of the carbon signals was consistent with the resonances observed in the \textsuperscript{1}H NMR experiment as well as the \textsuperscript{1}J \textsuperscript{1}H-\textsuperscript{13}C correlations observed in the HSQC-DEPTQ spectrum. In the \textsuperscript{1}H-\textsuperscript{1}H COSY spectrum, a chain of vicinal correlations observed from H-2 to H-14 (Fig. 2), which helped to construct one part of the compound structure as CH\textsubscript{3}-CH=CH-CH=CH=CH=CH\textsubscript{2}-CH\textsubscript{2}-CH\textsubscript{2}-CH\textsubscript{2}-CH=CH-CH=CH\textsubscript{2}. In the HMBC spectrum, correlations from δ1.18 (H-3’ and H-4’) to carbon signals at δ51.1 (C-1’), 71.6 (C-2’) and 27.2 (C3’ or C-4’), and from δ 3.27 (s, H-1’) to C-2’, C-3’ and C-4’ identified the other part of the molecule as 2-hydroxy-isobutyl moiety. Further correlations in the HMBC
spectrum from H-1’ (δ3.27), H-2 (δ5.99) and H-3 (δ7.12) to C-1 (δ169.4), in addition to the appearance of the methylene of the isobutyl moiety at δ55.1, established that the 2-hydroxy-isobutyl moiety was linked to the nitrogen of the amide group and the aliphatic moiety to the carbonyl of the amide. The geometry of the double bond C2/C3 was deduced as trans (JH2/H3= 15.0 Hz) like those of C4/C5, C8/C9 and C10/C11. Compound 1 did not show any optical activity suggesting that it was obtained as a racemic mixture. Thus compound 1 was identified as (12RS, 13RS)-(2E, 4E, 8E, 10E)-N-(2-hydroxy-2-methylpropyl)-12,13-dihydroxy-2,4,8,10-tetradecatetraenamide, given the trivial name zanthoamide G (1). The structure of zanthoamide E (1) resembled that of zanthoamide C isolated from *Zanthoxylum bungeanum* (Wang et al., 2016) with the only difference being the additional olefinic bond between C3 and C6 present in 1. Furthermore, the structure of 1 was supported by the fragmentation pattern observed in the EIMS experiment. The peaks corresponding to [M-C2H5O]+ and [M-H2O-C2H5O]+ could be attributed to the loss of the hydroxylated fragments on the aliphatic side on the molecule while peaks matching [M-CH3]+, [M-H2O]+ and [M-C2H5]+ could be characteristic for the loss of fragments on the 2-hydroxy-isobutyl moiety.

The molecular formula of compound 2 was determined as C18H29NO4 from its HRESIMS spectrum, where a pseudomolecular ion was observed as at m/z 346.1992 [M + Na]+ (calculated 346.1989 for C18H29NO4Na). The 1H and 13C NMR spectra (Table 1) of 2 established it as an aliphatic amide, similar to 1, with the only difference being the placement of the oxymethines, which were not adjacent to each other like in 1. A 1H-1H COSY spectrum revealed scaler couplings between oxymethine at δ4.28 (H-13) and methyl at δ1.20 (H-14) and also with the olefinic proton at δ5.73 (H-12), and the correlation from the other oxymethine at δ4.10 (H-8) to the methylene at δ1.60 (H-7) and the olefinic proton at δ5.68 (H-9) (Fig. 2). These correlations could be further confirmed from the HMBC spectrum, where correlations from H-8 to C-6 (δ29.9), C-7 (δ37.4) and C-9 (δ137.0), and from H-13 to C-12 (δ138.6) and C-14 (δ23.5) were observed (Fig. 2). The geometry of the olefinic bonds were confirmed as trans from relevant coupling constants ($J_{H2/H3}$= 15.1, $J_{H4/H5}$= 15.2, $J_{H9/H10}$= 14.6 and $J_{H11/H12}$= 14.5). Compound 2 was optically inactive suggesting that it was a racemic mixture. Thus, compound 2 was identified as (8RS, 13RS)-(2E, 4E, 9E, 11E)-N-(2-hydroxy-2-methylpropyl)-8,13-dihydroxy-2,4,9,11-tetradecatetraenamide, and given the trivial name, zanthoamide H (2). The structure of zanthoamide H (2) is quite similar to that of zanthoamide B isolated from *Z. bungeanum* (Wang et al., 2016) with the only difference being the hydroxyl groups are in positions 8 and 13 in 2, whilst in zanthoamide B, they are in positions 6 and 13. It should be
noted that in the published paper (Wang et al., 2016) the IUPAC name given to zanthoamide B was erroneous and should be reviewed to (6RS, 13RS)-(2E, 4E, 9E, 11E)-N-(2-hydroxy-2-methylpropyl)-6,13-dihydroxy-2,4,9,11-tetradecatetraenamide to comply with the corresponding discussion and published spectroscopic data supporting the two –OH groups in that molecule to be in positions 6 and 13.

Compound 3 has a molecular formula of C₁₈H₂₇NO₄, determined from the pseudomolecular ion [M + H]^+ observed at m/z 322.2017 in its HRESIMS (calculated 322.2013 for C₁₈H₂₈NO₄). ^{1}H and ^{13}C NMR data (Table 1) of 3 indicated that like 1 and 2, it was also an aliphatic amide, and there were spectral similarities with compound 2, with the obvious difference being the absence of the C-8 hydroxyl group as observed in 2, and the presence of a ketone group in 3 instead, which was confirmed by the signal at δ202.0 in the ^{13}C NMR spectrum (Table 1). The position of the ketone group was confirmed from the HMBC experiment, where correlations were observed between the ketone carbonyl carbon (δ202.0) and the protons at δ2.48 (H-6), 6.21 (H-9), 2.79 (H-7) and 7.29 (H-10) (Fig. 2). This was also supported by the downfield shifts of C-10 and C-12 from δ129.8 and 138.6 in 2 to δ144.5 and 149.3, respectively, in 3, caused by the presence of a ketone group nearby. The geometry of the double bonds C2/C3, C4/C5, C9/C10 and C11/C12 was determined as trans from the relevant coupling constants, J = 15.0, 15.0, 15.6 and 15.1, respectively. Unlike compounds 1 and 2, compound 3 was found to be optically active, [α]D^25 = -25.6. However, the absolute configuration could not be determined because of paucity of this sample. Thus, compound 3 was identified as (13*)-(2E, 4E, 9E, 11E)-N-(2-hydroxy-2-methylpropyl)-13-hydroxy-2,4,9,11-tetradecatetraenamide and given the trivial name, zanthoamide I (3).

This is the first report on the occurrence of compounds 4, 6-8 and 10, in addition to three new alkamides (1-3), in Z. zanthoxyloides. Several alkamides with structures similar to zanthoamines G-I (1-3) were previously reported in several Zanthoxylum species including Z. achtoum, Z. ailanthoides, Z. armatum, Z. bungeanum, Z. heitzii, Z. integrifoliolum, Z. piperitum, Z. schinifolium, Z. syncarpum, Z. tessmannii and Z. zanthoxyloides (Ross et al., 2005; Wang et al., 2016; Chruma et al., 2018). Araliopsine (4) was previously isolated from Z. simulans (Chang et al., 1981), N-methylplatydesminium catium (6) from Z. usambarense and Z. chalybeum (Kato et al., 1996), isoplatydesmine (7) from Z. nididum (Ishikawa et al., 1995), myrtopsine (8) from Z. integrifoliolum (Ishii et al., 1982) and N-methylatanine (10) from Z. beecheyanum (Cheng et al., 2004) and Z. rigidum (Moccellin et al., 2009).
Compounds 1-13 were evaluated for their cytotoxicity against A549 (adenocarcinomic human alveolar basal epithelial), MCF7 (human breast adenocarcinoma), PC3 (human prostate cancer) and PNT2 (human normal prostate epithelium) cells (Table 2). Among the tested compounds, hesperidin (13) showed a moderate level of cytotoxicity against A549, MCF7 and PC3 cells with IC\textsubscript{50} values of 29.5±7.5, 74.2±17.8 and 51.7±8.7 μM, respectively, with the IC\textsubscript{50} value for the normal cell line (PNT2) being 129.0±20.3 μM. Skimmianine (5) and sesamin (11) were moderately active against MCF7 and PC3 cells, exhibiting IC\textsubscript{50} values of 53.7±09.5 and 33.4±9.8 μM, respectively. None of the isolated new compounds (1-3) was particularly cytotoxic against the cell lines at the test concentrations (0-200 μM). However, only zanthoamide G (1) showed some cytotoxicity against MCF7 cells (IC\textsubscript{50} = 153.6±32.7μM).

Several alkamides isolated from the *Zanthoxylum* genus have been found to possess weak or moderate cytotoxicity (Devkota et al., 2012; Wang et al., 2017). Additionally, alkamides possess antidiabetic, anti-inflammatory, immunomodulatory and nerve growth factor potentiating properties (Greger, 2016; Tian et al., 2016; Wang et al., 2017).

3. Materials and methods

3.1. General experimental procedures

Analytical and preparative TLC were carried out on 0.2 mm Sigel 60 F\textsubscript{254} plates (Merck, Germany). Spots were visualized under short (254 nm) and long wavelength (366 nm), and also by spraying with a 1% anisaldehyde solution in aqueous H\textsubscript{2}SO\textsubscript{4} followed by heating to 105°C for 5 min. The NMR spectroscopic analyses were performed in CD\textsubscript{3}OD or CDCl\textsubscript{3} solution on a Bruker AMX300 instrument (300 MHz for \textsuperscript{1}H and 75 MHz for \textsuperscript{13}C) or Bruker AMX600 NMR spectrometer (600 MHz for \textsuperscript{1}H and 150 MHz for \textsuperscript{13}C). HRESIMS analyses were performed on a Xevo G2-S ASAP or LTQ Orbitrap XL1 spectrophotometer. EI\textsubscript{MS} analysis was recorded on a Finnigan MAT 95 spectrometer. Analytical HPLC was performed on a Dionex UPLC 3000 (Thermoscientific, UK) HPLC coupled with a photo-diode-array (PDA) detector (Thermoscientific). Extracts and fractions were analyzed on a Phenomenex C\textsubscript{18} column (150 × 4.6 mm, 5 μm, Phenomenex, USA). An Agilent 1200 Infinity series preparative HPLC system coupled with a PDA detector (Agilent, UK) was used to isolate compounds; a Hichrom ACE C18 preparative column (150 × 21.2 mm, 5 μm) was used. The column temperature was set at 25 °C. The chromatogram was monitor at variable UV–vis wavelengths.
(215, 254, 280 and 320 nm). Optical rotation was determined using Bellingham-Stanley ADP660 polarimeter (MeOH, c in g/100mL). UV spectra were recorded on Analytik Jena Specord 210 spectrophotometer. IR was recorded on an Agilent Cary 630 FT-IR.

3.2. **Plant material**

The fruits of *Z. zanthoxyloides* were collected from Dschang local market, Western Region, Cameroon, in November 2015, and identified by Mr Victor Nana, a taxonomist at the Cameroon National Herbarium, by comparing the plant sample with the voucher specimen 21793/SFR/CAM.

3.3. **Extraction and isolation of compounds**

The air-dried ground fruits (350.0 g) of *Z. zanthoxyloides* were Soxhlet-extracted, successively, with *n*-hexane, DCM and MeOH (800 mL, 10 cycles each). After evaporation at 40°C under reduced pressure, 34.6 g, 3.9 g and 19.6 g of *n*-hexane, DCM and MeOH extracts were obtained, respectively. A portion of the dried MeOH extract (2 g) was suspended in 10 mL of 10% MeOH- water and loaded on to a Strata C-18-E cartridge (Phenomenex, USA) (20 g), previously washed with MeOH (50 mL), followed by equilibration with water (100 mL). The cartridge was eluted with MeOH–water mixture of decreasing polarity to obtain four fractions: 20, 50, 80 and 100% MeOH in water (200 mL each) (F1-F4, respectively). All fractions were concentrated to dryness using a combination of rotary evaporator and freeze-dryer and stored at 4°C until further use. F3 (845.8 mg) was subjected to preparative HPLC using an ACE prep-column (150 × 21.2 mm, Hichrom Ltd, UK), flow rate 10 mL/min, mobile phase gradient of water (A) and methanol (B) both containing 0.1% TFA: 30-100% B, 0-30 min; 100% B, 30-35 min; 100-30% B, 35-40 min, monitored at wavelengths 254 and 280 nm to yield *N*-methylplatydesmine cation (12.1 mg) (6), myrtopsine (2.3 mg) (8), hesperidin (1.8 mg) (12), hesperetin (2.4 mg) (12), skimmianine (7.8 mg) (5), atanine (3.3 mg) (9), N-methylatanine (2.8 mg) (10) and sesamin (4.5 mg) (11) having the retention times (*t*<sub>R</sub>) 3.2, 7.5, 19.2, 24.8, 25.7, 29.3, 30.8 and 31.9 min, respectively.

F2 (704.8 mg) was also analyzed by preparative HPLC as above, but with a different mobile phase: a gradient of water containing 0.1% TFA (A) and acetonitrile (B): 30-100% B over 30 min and monitor at wavelengths 254 and 280 nm to yield *N*-methylplatydesmine cation (3.5 mg) (6) and isoplatydesmine (3.6 mg) (7) having the retention times (*t*<sub>R</sub>) 3.1 and 5.3 min,
respectively. Fraction F2-B (5.7 mg) collected at $t_R = 4.8$ min was further purified through preparative TLC to afford 2 (3.2 mg, EtOAc-MeOH 70:30, $R_f$ 0.41). Fraction F2-D (3.6 mg) collected at $t_R = 5.5$ min was also purified by preparative TLC to obtain 3 (1.9 mg, EtOAc-MeOH 70:30, $R_f$ 0.46). Preparative TLC of fraction F2-E (3.8 mg) collected at $t_R = 6.2$ min provided 1 (1.8 mg, EtOAc-MeOH 70:35, $R_f$ 0.41). Purification of fraction F2-F (10.2 mg) collected at $t_R = 6.5$ min using TLC with a mixture of EtOAc-MeOH (70:35) as eluent afforded more of 1 (2.5 mg, $R_f$ 0.41) and ariolipsine 4 (3.6 mg, $R_f$ 0.28).

3.4. **Zanthoamide G (1)**

White viscous liquid; $[\alpha]_D^{25} +0.0$ (c 0.004, MeOH); UV $\lambda_{\text{max}}$ (nm): 236, 260; FT-IR (ATR) $\nu_{\text{max}}$(cm$^{-1}$): 3380, 2920, 2850, 1580; HRESIMS $m/z$ 346.1984 [M + Na]$^+$ (calc 346,1989 for C$_{18}$H$_{29}$NO$_4$Na$^+$); EIMS $m/z$ (rel. int.): 278 (100) [M-C$_2$H$_5$O]$^+$, 260 (95) [M-H$_2$O-C$_2$H$_5$O]$^+$, 293 (5) [M-2xCH$_3$]$^+$, 305 (5) [M-H$_2$O]$^+$, 308 (2) [M-CH$_3$]$^+$; see Table 1 for $^1$H NMR (600 MHz, CD$_3$OD) and $^{13}$C NMR (150 MHz, CD$_3$OD) data.

3.5. **Zanthoamide H (2)**

White viscous liquid; $[\alpha]_D^{25} +0.0$ (c 0.006, MeOH); UV $\lambda_{\text{max}}$ (nm): 232, 262; FT-IR (ATR) $\nu_{\text{max}}$(cm$^{-1}$): 3325, 2950, 2840, 1680, 1600, 1535; HRESIMS $m/z$ 346.1992 [M + Na]$^+$ (calc 346,1989 for C$_{18}$H$_{29}$NO$_4$Na$^+$); EIMS $m/z$ (rel. int.): 323 (10) [M]$^+$, 306 (11) [M+H-H$_2$O]$^+$, 263 (27) [M-C$_3$H$_6$O]$^+$, 183 (45) [M+H-C$_8$H$_{13}$O$_2$]$^+$, 165 (65) [M+H-H$_2$O-C$_8$H$_{13}$O$_2$]$^+$; see Table 1 for $^1$H NMR (600 MHz, CD$_3$OD) and $^{13}$C NMR (150 MHz, CD$_3$OD) data.

3.6. **Zanthoamide I (3)**

White viscous liquid; $[\alpha]_D^{25} - 25.6$ (c 0.013, MeOH); UV $\lambda_{\text{max}}$ (nm): 214, 264; FT-IR (ATR) $\nu_{\text{max}}$(cm$^{-1}$): 3380, 2920, 2860, 2920, 2850, 1680, 1620, 1550, 980; HRESIMS $m/z$ 322.2017 [M+H]$^+$ (calc 322.2013 for C$_{18}$H$_{28}$NO$_4$); EIMS $m/z$ (rel. int.): 321 (15) [M]$^+$, 303 (17) [M-H$_2$O]$^+$, 273 (20) [M-C$_2$H$_5$O]$^+$, 263 (90) [M-C$_3$H$_6$O]$^+$; see Table 1 for $^1$H NMR (600 MHz, CD$_3$OD) and $^{13}$C NMR (150 MHz, CD$_3$OD) data.

3.7. **Cytotoxicity assay**

The *in vitro* antiproliferative (cytotoxic) activity of all the isolated compounds (1-13) from *Z. zanthoxyloides* was assessed against A549, MCF7 and PC3 human cancer cell lines, and also against normal prostate epithelium cells (PNT2). The cell lines were grown in RPMI medium supplemented with L-glutamine (2 mM), penicillin (100 U/mL), streptomycin (100
μg/mL) and 10% fetal bovine serum (FBS). The cells were cultured at 37°C, 5% CO₂ and 95% humidity and were seeded into 96 wells plate (1.2×10⁴/well) and incubated for 24h. The cells were then treated for 48h with the isolated compounds (0 to 200 µM) before the cell viability was assessed using the MTT assay (Mosmann, 1983). To achieve this, the medium in each well was replaced by MTT solution (500 µg/mL in medium) and incubated for 2h. The toxicity of the compounds was assessed by the ability of the cells to reduce the yellow MTT dye to blue formazan crystals. The formazan crystals formed were dissolved in DMSO and optical density was read at 570 nm in a ClarioStar plate reader. Three individual wells were assayed per treatment; the assay was repeated three times and cytotoxic activity was determined as percentage of control cells [(absorbance of treated cells/absorbance of untreated cells) × 100]. Doxorubicin was used as positive control and the IC₅₀ value of each test sample was calculated using the software Graphad Prism 7.02.
Acknowledgments

Mass spectrometry data were acquired at the EPSRC UK National Mass Spectrometry Service (NMSS) at Swansea University. We thank the Commonwealth Commission for the award of a PhD scholarship to S. T. G.

Appendix A. supplementary data

NMR and MS spectra of zanthoamides G-I (1-3).

References


Fig. 1. Structures of zanthoamides G-I (1–3)
Fig. 2. $^1$H-$^1$H COSY and some key HMBC correlations of zanthoamide G-I (1-3)
Table 1.

$^1$H (600 MHz) and $^{13}$C (150 MHz) NMR data of zanthoamides G-I (1-3)$^a$

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<td>6.20 m</td>
<td>6.42 dd (10.3, 15.1)</td>
</tr>
<tr>
<td>12</td>
<td>3.91 dd (6.4, 12.9)</td>
<td>5.73 m</td>
<td>6.31 m</td>
</tr>
<tr>
<td>13</td>
<td>3.58 m</td>
<td>4.28 q (6.3, 12.5)</td>
<td>4.38 m</td>
</tr>
<tr>
<td>14</td>
<td>1.11 d (6.3)</td>
<td>1.20 d (6.4)</td>
<td>1.22 d (6.5)</td>
</tr>
<tr>
<td>1’</td>
<td>3.27 s</td>
<td>3.28 s</td>
<td>3.27 s</td>
</tr>
<tr>
<td>2’</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3’</td>
<td>1.18 s</td>
<td>1.19 s</td>
<td>1.18 s</td>
</tr>
<tr>
<td>4’</td>
<td>1.18 s</td>
<td>1.19 s</td>
<td>1.18 s</td>
</tr>
<tr>
<td>NH</td>
<td>8.56 br s</td>
<td>8.56 br s</td>
<td>8.56 br s</td>
</tr>
</tbody>
</table>

$^a$Spectra obtained in CD$_3$OD, and DEPTQ, COSY, HSQC and HMBC experiments confirmed assignment of all $^1$H and $^{13}$C signals
Table 2
Cell Growth Inhibitory Activities (IC$_{50}$ in µM) of Isolated Compounds against Cancer (A549, MCF7, PC3) and Normal (PNT2) Cells* 

<table>
<thead>
<tr>
<th>Compounds</th>
<th>IC$_{50}$ values in µM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A549</td>
</tr>
<tr>
<td>1</td>
<td>&gt;200</td>
</tr>
<tr>
<td>2</td>
<td>&gt;200</td>
</tr>
<tr>
<td>3</td>
<td>&gt;200</td>
</tr>
<tr>
<td>4</td>
<td>&gt;200</td>
</tr>
<tr>
<td>5</td>
<td>113.4±15.8</td>
</tr>
<tr>
<td>6</td>
<td>&gt;200</td>
</tr>
<tr>
<td>7</td>
<td>&gt;200</td>
</tr>
<tr>
<td>8</td>
<td>&gt;200</td>
</tr>
<tr>
<td>9</td>
<td>112.0±17.4</td>
</tr>
<tr>
<td>10</td>
<td>114.7±18.2</td>
</tr>
<tr>
<td>11</td>
<td>108.5±22.1</td>
</tr>
<tr>
<td>12</td>
<td>151.4±25.4</td>
</tr>
<tr>
<td>13</td>
<td>29.5±7.5</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>1.3±0.3</td>
</tr>
</tbody>
</table>

*Data are represented as mean ± SEM (n = 3); IC$_{50}$ = sample concentration that caused 50% cell growth inhibition