Kingianins O–Q: Pentacyclic polyketides from *Endiandra kingiana* as inhibitor of Mcl-1/Bid interaction

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**Abstract**

A phytochemical study of the EtOAc-soluble part of the methanolic extract of the bark of *Endiandra kingiana* led to the isolation of three new pentacyclic kingianins as racemic mixtures, kingianins O–Q (1–3), together with the known kingianins A, F, K, L, M and N (4–9), respectively. The structures of the new kingianins 1–3 were determined by 1D and 2D NMR analysis in combination with HRESIMS experiments. Kingianins A–Q were assayed for Mcl-1 binding affinity. Kingianins G and H were found to be potent inhibitors of Mcl-1/Bid interaction. A structure–activity relationship study showed that potency is very sensitive to the substitution pattern on the pentacyclic core. In addition, in contrast with the binding affinity for Bcl-xL, the levorotatory enantiomers of kingianins G, H and J exhibited similar binding affinities for Mcl-1 than their dextrorotatory counterparts, indicating that the two anti-apoptotic proteins have slightly different binding profiles.

1. Introduction

Attention to targeted therapy has dramatically increased especially in cancer treatments. The term “targeted cancer therapy” refers to a new generation of drugs designed to interfere with a specific molecular target that is believed to play a critical role in tumour growth or progression and not expressed significantly in normal cells [1–3]. There has been a rapid increase in the identification of targets that have potential therapeutic application [4–5] and among them are the anti-apoptotic protein Mcl-1, which is overexpressed in many cancers, contributes to tumour progression, and has emerged as one of the major resistance factors in cancer cells [3,6]. Placzek and co-workers investigated the mRNA expression levels of all six antiapoptotic Bcl-2 subfamily members in 68 human cancer cell lines [7]. The study revealed that Mcl-1 represents the antiapoptotic Bcl-2 subfamily member with the highest mRNA level in the lung, prostate, breast, ovarian, renal, and glima cell lines [7].

Our group has previously isolated and characterized a series of new pentacyclic kingianin analogues, kingianins A–N, isolated from the EtOAc extract of the bark of *Endiandra kingiana* Gamble [8–9]. Among them, kingianin G showed good binding affinity to the antiapoptotic protein Bcl-xL [9]. This research focuses more on the investigation of the chemical composition of the MeOH extract because it showed a moderate potency as modulating agent with the Mcl-1/Bid (22% at 10 μg/mL). Herein, we report the isolation and structural elucidation of the new kingianins which belong to the new pentacyclic polyketide; kingianins O–Q (1–3), along with the known kingianins A, F, K, L, M and N (4–9) [8–9]. The Mcl-1 binding affinity of the kingianins was also communicated and this is the first report on this activity for the pentacyclic kingianin series.

2. Experimental

2.1. General experimental procedures

Optical rotations were measured using a JASCO P-1020 polarimeter. IR spectra ( neat) were recorded on a Perkin Elmer RXI FT-IR spectrometer. 1D (1H, 13C, DEPT) and 2D (COSY, NOESY, HSQC, HMBC) NMR experiments were carried out on a Bruker Avance 600 (600 MHz for 1H NMR, 150 MHz for 13C NMR) spectrometer. Data were analysed via TopSpin 3.0 software package. Chemical shifts were internally referenced to the solvent signals in CDC13 (1H δ 7.26; 13C δ 77.0). High-resolution ESIMS were recorded on a Thermoquest TLM LCQ Deca ion-trap mass spectrometer. Silica gels (230–400 mesh) (Merck) were used for column chromatography (CC), and silica gel 60 F-254 (Merck) was used for analytical TLC. A Waters® X-Bridge C18 column (250 × 10.0 mm, 5.0 μm); was used for semi-preparative HPLC separations employing a Waters auto purification system equipped with a sample manager (Waters 2767), a column fluidics organizer, a binary pump (Waters 2525), a UV–Vis diode array detector (190–600 nm, Waters 2996),

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and a PL-ELS 1000 ELSD Polymer Laboratory detector. 10% Vanillin in ethanol was used as the detecting reagent.

2.2. Plant material

The bark of E. kingiana Gamble was collected at Reserved Forest Sg. Temau, Kuala Lipis, Pahang, Malaysia in May 2006. This plant was identified by T. Leong Eng, a botanist in University of Malaya [15–17]. A voucher specimen (KL-5243) has been deposited at the Herbarium of the Department of Chemistry, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia.

2.3. Extraction and isolation

The air-dried bark of E. kingiana (1.5 kg) was sliced, ground and extracted with EtOAc (3 × 1.5 L) followed by MeOH (3 × 1.5 L) at 40 °C and 100 bar using a Zipptex static high pressure, high temperature extractor developed at the ICSN pilot unit. The solvents were removed under reduced pressure to give residues of 20.0 g (EtOAc) and 225.0 g (MeOH).

2.3.1. Kingianin O (1)

White powder, [α]D25 ± 0 (c 0.20, CHCl3), UV λmax (MeOH) nm (log ε): 233 (3.03), 286 (3.12), IR νmax (neat) 3297, 1718, 1625, and 1540 cm⁻¹, HRESIMS (negative ion mode) m/z 650.3047 [M–H]⁻ (calcd for C38H42NO7; m/z 650.3181), 1H and 13C NMR: see Table 1.

2.3.2. Kingianin P (2)

White powder, [α]D25 ± 0 (c 0.12, CHCl3), UV λmax (MeOH) nm (log ε): 233 (3.21), 286 (3.30), IR νmax (neat) 3297, 1725, 1630, and 1541 cm⁻¹, HRESIMS (positive ion mode) m/z 624.2928 [M + H]⁺ (calcd for C38H42NO7; m/z 624.2961), 1H and 13C NMR: see Table 1.

2.3.3. Kingianin Q (3)

White powder, [α]D25 ± 0 (c 0.20, CHCl3), UV λmax (MeOH) nm (log ε): 233 (3.03), 286 (3.12), IR νmax (neat) 3297, 1718, 1625, and 1540 cm⁻¹, HRESIMS (negative ion mode) m/z 650.3047 [M–H]⁻ (calcd for C40H44NO7; m/z 650.3181), 1H and 13C NMR: see Table 1.

2.4. Mcl-1 binding affinity

The binding affinities of compounds 1–3 and known kingianins for McI-1 was evaluated by competition against fluorescently labelled reference compounds, Bid, as described by Qian et al. (2004) [11]. Mouse DN150/D25 McI-1 protein was recombinant produced by N. Birlirask at ICSN. Bid and 5-carboxyfluorescein-Bid peptides were synthesized by PolyPeptide Laboratories (Strasbourg, France). All sequences are available in Azmi et al. (2014) [10]. Unlabelled peptides were dissolved in DMSO (Carlo Erba, Val de Reuil, France) and labelled peptides were diluted in assay buffer, which contained 20 mM Na2HPO4 (pH 7.4), 50 mM NaCl, 2 μM EDTA, 0.05% Pluronic F-68, without pluronic acid for storage at −20 °C. Liquid handling instrument, Biomek®NX and Biomek®3000 (Beckman Coulter, Villepinte, France), were used to add protein and fluorescein-labelled peptides. 15 nM labelled BH3 peptide, 100 nM protein, and 100 μM of unlabelled BH3 peptide or compound (first diluted in 10 mM DMSO and then buffer to a final concentration from 10⁻⁸ to 10⁻⁴ M) into a final volume of 40 μL were distributed in a 96 well black polystyrene flat-bottomed microplate (VWR 734–1622). The microplate was then incubated at room temperature for 1 h and shaken before the fluorescent polarization measure. Fluorescence polarization in millipolarization units was measured with a Beckman Coulter Paradigm® using a FP cartridge (λex 485 nm, λem 535 nm). The exposure time was 300 ms per channel. All experimental data were collected using the Biomek Software® (Beckman Coulter, Inc., Brea, CA, USA) and analysed using Microsoft Excel 2010 (Microsoft, Redmond, WA, USA). Results are expressed in terms of binding activity, i.e., Ki, the concentration corresponding to 50% of such inhibition, and corrected for experimental conditions according to the Kenakin rearranged equation [12], which was adapted from Cheng and Prusoff equation [13]. Meiocongin A and unlabelled peptide Bid were used as positives control. The performance of the assays was monitored by the use of Z' factors as described by Zhang et al., [14]. The Z' factors for this assay was 0.7 (Mcl-1/Bid) indicating that they should be robust assays.

3. Results and discussion

The EtOAc-soluble part of the methanol extract of E. kingiana was subjected to silica gel chromatography to afford eight fractions 1–8. Fractions Fr.5–Fr.7 were further purified using silica gel column as well as semi-preparative HPLC (C-18) leading to the isolation of three new kingianins; kingianin O–Q (1–3), together with kingianins A, F, K, L, M and N (4–9) (Fig. 1). The assignments of the 1H and 13C NMR data were then established through in depth analysis of the 2D NMR; NOESY, COSY, HSQC and HMBC experiments. All compounds 1–9 were isolated as optically inactive compounds, thus suggesting that they were racemic mixtures.

Kingianin O (1) was obtained as a white powder. Its HRESIMS indicated a pseudomolecular ion peak [M + H]⁺ at m/z 624.2954; suggesting a molecular formula of C38H42NO7 (calcd for C38H42NO7; m/z 624.2961); from which 19° of unsaturation were deduced. The IR spectrum of 1 showed the absorption bands at νmax 3295, 1718, 1624, and 1542 cm⁻¹ corresponding to the N–H amide elongation, C=O stretching of an acid amide, and N–H amide deformation, respectively thus indicating the presence of a carboxylic acid group and an amide group.

The 1H NMR (600 MHz, CDCl3) spectra of 1 showed a set of multiplets between δH 1.25 and 2.60 corresponding to twelve protons of the pentacyclic skeleton which is the characteristic feature of the kingianin series [8–9]. Correlations observed from the 1H–1H COSY and...
HSQC spectra revealed two sets of eight contiguous structural sequence involving (H-1 [δH 2.18 (m), H-2 [δH 2.46 (m)], H-3, H-4, H-5 [δH 2.06 (m)], H-6 [δH 1.26 (d, J = 9.5 Hz), H-7 [δH 1.86 (m)], H-8 [δH 1.70 (m)]], and (H-1′ [δH 1.84 (m)], H-2′ [δH 2.09 (m)], H-3′ [δH 2.08 (m)], H-4′, H-5′, H-6′ [δH 2.50 (m)], H-7′ [δH 2.49 (m)] and H-8′ [δH 2.46 (m)]. The pentacyclic rings were fused at the C-2′-C-7, C-5′-C-6, C-6′-C-3′ and C-2′-C-7′ junctions. The HMBC correlations from H-6/C-2-C, C-8, C-4′ and C-6′, H6/C-2′-C-2′-C-3′ and C-2′-C-7′ established the pentacyclic skeletal structure of I consisting of two four-membered rings and three six-membered rings. In addition, four cis-form vinyl proton signals at δH 5.48 (dd, J = 9.7, 9.5 Hz, H-3), 5.60 (dd, J = 9.7, 9.5 Hz, H-4), 6.00 (dd, J = 7.6, 7.1 Hz, H-4′), and 6.10 (t, J = 7.1 Hz, H-5′) (Table 1).

Meanwhile, the presence of two 1,3,4-trisubstituted benzene moieties was suggested by a doublet at δH 6.60 (d, J = 1.1 Hz, H-11, H-11′) and the two ortho-meta-coupled doublets at δH 6.69 (d, J = 8.1 Hz, H-14, H-14′) and 6.53 (dd, J = 8.1, 1.1 Hz, H-15, H-15′). In addition, the two singlets correspond to two protons each at δH 5.93 and 5.94 (H3-16 and H3-16′, respectively) confirmed the presence of the methylenedioxy group. An N-ethylacetamide group presence was imputed from the observation of a doublet of quartets representing two protons at δH 3.22 (J = 5.7, 7.1 Hz, H-20′), a triplet corresponding to a methyl group at δH 1.10 (J = 7.1 Hz, H-21′) and another triplet at δH 5.36 belonging to NH-19′ (J = 5.7 Hz). The COSY spectrum showed correlations corresponding to the NH-19′-H2-20′-H2-21′ spin system; H-19′/H2-20′, H2-20′/H2-21′.

The 13C NMR and DEPT spectra displayed 38 carbon resonances; one methyl, 7 methylenes, of which two were methylenedioxy, 22 methines, and 8 quaternary carbons including two carbonyl groups which resonated at δC 178.0 (C-18, acetic acid moiety) and 172.6 (C-18′, N-ethylacetamide moiety). Resonances of the sixteen carbons involved in the pentacyclic system of kingianins appeared as twelve sp3 methine carbons at δC 184.3 (C-24, C-24′), 184.5 (C-25, C-25′), 190.4 (C-26, C-26′) and 190.5 (C-27, C-27′) as observed in DEPT spectrum.

Finally, the connectivities between the four substituents and the pentacyclic skeleton were established by the HMBC and COSY correlations (Fig. 2). The connectivity of the N-ethylacetamide group to the core skeleton was confirmed by the correlations between H-8′ and H2-17′ in the COSY spectrum, and between H-8′ and C-17′ in the HMBC spectrum indicated that this group is attached to C-8′. In addition, the COSY spectrum showed cross peaks between the methylene protons (H2-17) with H-1, therefore indicating that the acetic acid is linked to the pentacyclic core at C-1. Finally, the location of the two methylenedioxybenzyl groups at C-8 and C-8′ respectively were...
deduced from the correlation between H-8/H2-9 and H-1′/H2-9′, and the HMBC cross peaks observed between C-9/C-9′ with H-11/H-11′ and H-15/H-15′. (See Fig. 3.)

The relative configuration at C-1, C-8, C-1′ and C-8 where the four substituents are attached could be deduced from the NOESY experiment. The cross peaks between H-6/H-8 and H-2′/H-8′ indicated that the benzyl group at C-8 and the amide group at C-8′ were in the β-position. Meanwhile, the NOESY correlations between H-7/H-1 and H-4′/H-1′ suggested that the acetic acid group at C-1 and benzyl group at C-1′ were in the α-position. All physicochemical data were in full agreement with the proposed structure of 1 as shown, which was named kingianin O.

Kingianin P (2) was obtained as a white powder. Its HRESIMS showed a pseudomolecular ion peak [M + H]⁺ at m/z 624.2928, corresponding to the molecular formula of C38H42NO7 (calcld. for C38H42NO7; m/z 624.2961). IR absorption bands at νmax 3297, 1725, 1630, and 1541 cm⁻¹ corresponding to the N–H amide elongation, C=O stretchings of an acid and amide, and N–H amide deformation. The NMR data for 2 was reminiscent to those of 1 (Table 1), with the same pentacyclic skeleton but differing in the locations of the acetic acid, the N-ethylacetamide.
and the two methylenedioxybenzyl moieties. The $^1$H–$^1$H COSY spectrum of 2 revealed the following cross peaks: H-1/H$_2$-9 and H-8′/H$_2$-9′, allowing the placement of the two methylenedioxybenzyl groups at C-1 and C-8′. In addition, the HMBC correlations between H$_2$-9 with C-11 ($\delta_C$ 108.3) and C-15 ($\delta_C$ 121.0), and that of H$_2$-9′ with C-11′ ($\delta_C$ 108.3) and C-15′ ($\delta_C$ 121.0), further verified the connectivities of the methylenedioxyphenyl groups at C-1 and C-8′, respectively. Meanwhile, the presence of the H-8–H$_2$-17 spin system as evidenced from the COSY correlations, and the HMBC correlations between H$_2$-17 with C-8 and C-18 carbonyl carbon ($\delta_C$ 178.2), confirmed that the acetic acid chain is attached to C-8. The N-ethylacetamide group is located at C-1′, as suggested from the COSY correlations between H-1′ and H$_2$-17′, and the HMBC correlations between H$_2$-17′ with C-1′, C-2′, C-8′ and the C-18′ carbonyl carbon ($\delta_C$ 171.6). The NOESY correlations between H-2′/H-8′ and H-4′/H-1′.

**Fig. 2.** Key $^1$H–$^1$H COSY (bold) and HMBC ($^1$H → $^{13}$C) correlations of 1–3.

**Fig. 3.** Key NOESY ($^1$H ↔ $^1$H) correlations of compound 1–3.
Hence, the structure of ed a pseudomolecular ion peak \([M+H]^+\) for compounds 1 was elucidated as shown, and was named kingianin P.

Kingianin Q (3) was obtained as a white powder. Its HRESIMS indicated a pseudomolecular ion peak \([M+H]^+\) at \(m/z 650.3047\); suggesting a molecule formula of \(C_{36}H_{42}NO_8\) (calcld. for \(C_{36}H_{42}NO_8\), \(m/z 650.3118\)), indicating that a compound 3 possess two additional CH2 groups compare to 1. The presence of N-ethylacetamide and butanoic acid groups was supported by the IR spectrum which had absorption bands at \(\nu_{max} 3296\) (NH stretch), 1718 (C\(-\)O amide), and 1747 (C\(-\)O amide), and 1540 (NH bend- ing) \(\text{cm}^{-1}\). The \(^{13}\text{C}\) NMR spectra showed signals for two quaternary carbons at \(\delta_1 172.6\) and 178.1 (Table 1), consistent with one amide and one carboxylic functionality. The HMBC correlations of H-2\(_7\)-C-1, H-2\(_7\)-C-18 and H-2\(_{20}\)-C-18 confirmed the presence of the N-ethylacetamide substituent which is attached to the skeleton at C-1. Meanwhile, the H-8’-H-17’-H-18’-H-19’ spin systems inferred from the COSY spectrum, and the HMBC correlations between H-17’ with C-8’, and H-19’ with C-18’ and the C-20 carbonyl carbon \(\delta_1 178.1\), confirmed the connectivity of the butanoic acid chain to C-8’. In the HMBC spectrum, the cross peaks observed between C-9 (C-9’) with H-11 and H-15 (H-11’ and H-15’) and COSY correlations between H-9 and H-8, and H-9 and H-1’, confirmed that the two methyleneoxybenzyl moieties were located at positions C-8 and C-1’, respectively. Finally, the relative configuration of all of the stereocenters in 3, deduced from the NOESY experiment, and it is similar to compound 1. The cross peaks between H-4’-H-1’, and H-2’-H-8’ indicated that H-1’ is \(\alpha\)-oriented while H-8’ is \(\beta\)-oriented. The NOESY correlations between H-7’-H-1 and H-6’-H-8, indicated an \(\alpha\)-orientation of the amide chain at C-1, and \(\beta\)-orientation of the benzyl moiety at C-8. Thus, the structure of 3 was elucidated as shown, and was named kingianin Q.

Kingianins A–N (4–9) were readily identified by comparison of their spectroscopic data with literature data [8–9].

The Mcl-1/Bid assay was developed and established in 2013 in our laboratory [10]. The binding affinity to anti-apoptotic protein; Mcl-1 of compounds 1–3 together with known kingianins A–N were screened using fluorescence polarization assays according to Qian et al. (2004) [11]. The assays were based on the interaction of fluorescein-labelled peptides [the BH3 domain of BID protein (F-BID) to Mcl-1]. The results were given by the \(K_i\) values. (Table 2). Based on these results, \(\alpha\)-kingianins G, H, J, K and L exhibited similar binding affinities with their respective \(\beta\)-counterparts. It should be noted that \(\alpha\) and \(\beta\)-kingianins G, H, J and K exhibited the most pot binding affinities for the protein Mcl-1 with \(K_i\) values between 2 and 4 \(\mu\)M, while \(\alpha\)- and \(\beta\)-kingianins K and L were less active \((13 < K_i < 17 \mu\)M\), thus suggesting that the two acidic chains located at either positions 1 and 8’ or 1’ and 8 on the pentacyclic core would be essential for a significant binding affinity (due to insufficient amounts, \(\alpha\)- and \(\beta\)-kingianin I were not tested). Meanwhile, the stereochemistry of the core skeleton does not play such a vital role for binding affinity towards Mcl-1.

Conflict of interest

The authors declare no conflict of interest.

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