Glycopentanolones A–D, four new geranylated quinolone alkaloids from *Glycosmis pentaphylla*

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ABSTRACT

The ethanolic extract obtained from the stems of *Glycosmis pentaphylla* was found to suppress antigen-mediated degranulation of rat basophilic leukemia (RBL-2H3) cells. Four new geranylated 2-quinolone alkaloids, named glycopentanolones A–D (1–4), and 12 known metabolites (5–16) were isolated from the ethanolic extract from the stems of *G. pentaphylla* using bioassay-guided fractionation. Their structures were elucidated by a combination of 1D and 2D NMR, and HRESI-MS. The inhibitory effects of the isolated constituents on β-hexosaminidase release from RBL-2H3 cells were examined, and compounds 1, 5, 8 and 11 exhibited potent inhibitory activity with IC₅₀ values between 0.05 and 4.28 μM.

1. Introduction

*Glycosmis*, from the family Rutaceae, is a genus of approximately 50 species of glabrous shrubs that are distributed in warm and temperate regions worldwide including eastern, southern, and southeast Asia, and northeastern Australia [1]. Previous phytochemical reports indicated that the genus *Glycosmis* is a rich source of various types of alkaloids (including quinolones, quinazolines, furoquinolines, acridones, sulfur-containing amides and carbazole types), and many of these alkaloids possess anti-HIV, antibacterial, antitumor, DYRK1A-inhibiting, and antiproliferative activities [1–6]. *G. pentaphylla* (Retz.) DC. is used in traditional/folklore medicine to treat protracted diseases, soreness, numbness, fever, liver complaints, and other specific diseases [7,8]. The extracts and chemicals from the leaves and stems of *G. pentaphylla* have been recently reported to exhibit hepatoprotection, anti-inflammatory, antifungal, antihyperlipidemic, antioxidant, antipyretic, wound healing, insecticidal and larvicidal, and anthelmintic activities [9–16]. Recently, many reports of phytochemical investigations performed on this plant have been published. Some of the major classes of compounds reported from *G. pentaphylla* include alkaloids, flavonoids, phenolic derivatives, naphthoquinone, amides, imides, triterpenoids, and essential oils, which exhibit diverse biological effects, such as antibacterial, anti-tumor, antiproliferative, and anti-hepatocellular carcinoma (HCC) activities [3,17–28].

Allergy is a ‘hypersensitivity’ reaction that occurs mainly in response to environmental stimuli such as foreign proteins and allergens (dust mite, pollens, animal dander, and foods). Allergy is generally classified into four types, and type I allergy is the most common allergic reaction associated with asthma, allergic dermatitis, and other atopic disorders [29,30]. Type I hypersensitivity is characterized by IgE-mediated reactions. The clinical manifestations of type I allergic diseases are caused by the release of proinflammatory mediators, such as histamines and prostaglandins from IgE-sensitized effector cells when cell-bound IgE antibodies interact with the allergen [31]. When granules in basophils or mast cells activate, β-hexosaminidase is usually secreted along with histamine; this enzyme is thus used as a biological marker for allergy and immunological research in rat basophilic leukemia 2H3 (RBL-2H3) cell line [32,33]. Therefore, inhibition of the release of β-hexosaminidase in RBL-2H3 cells has been used commonly as a reliable parameter to predict possible antiallergic activities of natural sources [34,35]. In a search to discover anti-allergic metabolites from natural products, an n-hexane and EtOAc-soluble part of the...
ethanol extract of the stems of *G. pentaphylla* showed moderate promoting effects (IC_{50} value of 7.6μg/mL) on the release of β-hexosaminidase in the RBL-2H3 cells. The investigation to obtain bioactive compounds led to the isolation of four new geranylated 2-quinolone alkaloids (glycopentanolones A–D, 1–4) and 12 known compounds (5–16) (Fig. 1). Herein, the isolation and structural elucidation of these compounds as well as their inhibitory effects on the release of β-hexosaminidase in the RBL-2H3 cells are described.

2. Materials and methods

2.1. General experimental procedures

The UV and IR spectra were recorded with JASCO V-550 and JASCO FT/IR-4100 spectrometer (Tokyo, Japan). NMR spectra were obtained using a Bruker Ascend III 700 (CryoProbe) spectrometer (Rheinstetten, Germany) with tetramethylsilane as the internal standard, with chemical shifts expressed in δ values. Electrospray ionization (ESI) mass spectra were obtained using an LTQ Orbitrap XL (Thermo Scientific) and Triple TOF 5600+ mass spectrometer (AB SCIX, USA). Open column chromatography was performed using silica gel (Kiesel gel 60, 70–230 mesh and 230–400 mesh, Merck) and a Lichroprep RP-18 column (12nm S-7μm, YMC GEL, Japan). Thin layer chromatography (TLC) was performed using pre-coated silica gel 60 F254 (0.25mm, Merck) and pre-coated silica gel 60 RP-18F-254S (0.25mm, Merck) plates. Preparative HPLC was performed on a Shimadzu system (LC-8A pump and SPD-20A UV/VIS detector) using a YMC-Pack ODS A column (250 × 20 mmL.D.), and a mixed solvent system of MeCN–H_{2}O at a flow rate of 12 mL/min. Compound 48/80, ketotifen, 2,4-dinitrophenol (DNP)-specific monoclonal IgE, 2,4-dinitrophenylated bovine serum albumin (DNP-BSA) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, U.S.A). All chemicals and solvents were of analytical grade and used without further purification.

2.2. Plant material

Dried stems of *G. pentaphylla* were collected from Seima Protection Forest of Cambodia in September of 2016. One of the authors (Dr. J.S. Kang, National Institute of Biological Resources) performed the botanical identification, and a voucher specimen (#189) was deposited at the Bio-Center, Gyeonggido Business & Science Accelerator (GBSA), Suwon, Republic of Korea.

2.3. Extraction and isolation

The dried stems (5 kg) of *G. pentaphylla* were extracted with 70% ethanol (3 × 18 L) at room temperature. The combined ethanol extracts were then concentrated in vacuo at 40 °C to yield 500 g of residue. The ethanol extract was suspended in distilled water followed by partitioning with n-hexane, CH_{2}Cl_{2}, EtOAc, and n-BuOH. The n-hexane (IC_{50} < 1.5μg/mL) and EtOAc (IC_{50} 10.4μg/mL) soluble fractions showed an inhibitory activity when antigen-induced β-hexosaminidase was released from RBL-2H3 cells and was thus subjected to further isolation. The n-hexane-soluble layer (9.1 g) was separated using silica gel column chromatography (CC) eluted with n-hexane–CH_{2}Cl_{2} (1:1 to 0:1) and CH_{2}Cl_{2}–MeOH (1:0 to 5:1) step gradient to yield 16 fractions (#189-16-1 to #189-16-16) based on TLC analysis. Fraction #189-16-11 was further passed through a RP-18 MPLC column (RediSep® Rf silica gold 100g, 50ml/min) eluted with MeCN–H_{2}O (10:90 to 100:0, 60 min) gradient to yield ten fractions (#189-18-1 to #189-18-10). Compounds 2 (6.5 mg) and 4 (1.2 mg) were isolated from the above subfraction, #189-18-4, by preparative HPLC (MeCN–H_{2}O, 55:45 to 70:30, 40 min). Purification of subfraction #189-16-15 under the same HPLC conditions (MeCN–H_{2}O, 40:60 to 70:30, 40 min) yielded compounds 1 (18.6 mg), 3 (1.1 mg), 5 (13.6 mg), and 6 (8.1 mg). Fraction #189-18-15 was subjected to further chromatographic separation on a silica gel column and eluted with a step gradient of CH_{2}Cl_{2}–MeOH (90:1 to 5:1) to yield three subfractions (#189-17-1 to #189-17-3). Using the above HPLC system, compound 7 (12.4 mg) was obtained from #189-17-2 (MeCN–H_{2}O, 40:60 to 70:30, 40 min). The fraction #189-18-8 formed a precipitate when concentrated. The precipitate was washed repeatedly with CH_{2}Cl_{2}, followed by subsequent repeated washing with MeOH to obtain compound 8 (86.6 mg). The EtOAc-soluble fraction (30 g) was chromatographed on a RP-18 column and eluted with a MeOH–H_{2}O gradient system (20:80 to 100:0) to give six fractions (#189E-1 to #189E-6). Fraction #189E-2 was purified by preparative HPLC (MeCN–H_{2}O, 10:90 to 50:50, 30 min) to yield compounds 13 (13.5 mg), 14 (33.1 mg), and 15 (15.0 mg). Fraction #189E-
3 was subjected to MPLC over ODS (RediSep® Rf silica gold 150 g, 75 ml/min) eluting with a gradient of 25–100% (65 min) MeCN in H₂O to give five subfractions (#189E-3M-1 to #189E-3M-5). Compounds 10 (17.9 mg) and 12 (13.4 mg) were obtained from #189E-3M-1 (MeCN–H₂O, 15:85 to 40:60, 40 min), and compounds 9 (2.7 mg), 11 (2.2 mg), and 16 (4.2 mg) obtained from #189E-3M-2 (MeCN–H₂O, 13:87 to 40:60, 40 min) using the same HPLC system.

**Glycopentanolone A** (1): pale brown gum; UV (MeOH): λmax (log ε) 229 (4.62), 269 (3.78), 278 (3.75), 318 (3.89), 331 (3.70) nm; IR (KBr) νmax 3283, 2923, 2852, 1738, 1610, 1505, 1074, cm⁻¹; ¹H NMR (CDCl₃, 700 MHz) and ¹³C NMR (CDCl₃, 175 MHz) data, see Table 1; ESI-MS: m/z 344 [M+H]+, 366 [M+Na]+; HRESIMS (positive ion mode) m/z 312.1949 [M+H]+, calcld for C₂₀H₂₆NO₄, 312.1958.

**Glycopentanolone B** (2): pale brown gum; UV (MeOH): λmax (log ε) 229 (4.65), 269 (3.83), 278 (3.79), 316 (3.92), 330 (3.73) nm; ¹H NMR (CDCl₃, 700 MHz) and ¹³C NMR (CDCl₃, 175 MHz) data, see Table 1; ESI-MS: m/z 344 [M+H]+, 366 [M+Na]+; HRESIMS (positive ion mode) m/z 344.1853 [M+H]+, calcld for C₂₀H₂₆NO₄, 344.1856.

**Glycopentanolone C** (3): pale brown gum; UV (MeOH): λmax (log ε) 229 (4.69), 269 (3.87), 278 (3.85), 316 (3.95), 329 (3.77) nm; ¹H NMR (CDCl₃, 700 MHz) and ¹³C NMR (CDCl₃, 175 MHz) data, see Table 1; ESI-MS: m/z 328 [M+H]+, 350 [M+Na]+; HRESIMS (positive ion mode) m/z 328.1902 [M+H]+, calcld for C₂₀H₂₆NO₄, 328.1907.

**Glycopentanolone D** (4): pale brown gum; UV (MeOH): λmax (log ε) 229 (4.77), 269 (3.89), 278 (3.97), 316 (3.99), 330 (3.82) nm; ¹H NMR (CDCl₃, 700 MHz) and ¹³C NMR (CDCl₃, 175 MHz) data, see Table 1; ESI-MS: m/z 344 [M+H]+, 366 [M+Na]+; HRESIMS (positive ion mode) m/z 344.1853 [M+H]+, calcld for C₂₀H₂₆NO₄, 344.1856.

### 2.4. Assay of β-hexosaminidase release from RBL-2H3 cells and cytotoxicity

**Culturing of RBL-2H3 cells.** We selected the basophilic leukemia cell line, RBL-2H3, for use in this study. RBL-2H3 cells were grown in minimum essential medium (MEM) containing streptomycin (Wegene, Daegu, Korea), 15% fetal bovine serum (FBS), penicillin, and 2 mM glutamine at 37 °C in 5% CO₂.

**MTT assay for cell viability.** Cell viability was assessed using the 3-(4,5-dimethyl-2-y1)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay. RBL-2H3 cells were treated with MTT (5 mg/ml) in serum-free DMEM. After 3 h of incubation at 37 °C and 5% CO₂, 100 μL of 100% DMSO was added to the wells and the insoluble purple formazan product dissolved to form a colored solution. We then used an ELISA reader to measure the absorbance of the solution at 540 nm.

**Inhibition of β-hexosaminidase release from RBL-2H3 cells.** RBL-2H3 cells were dispensed in a 24-well plate with 5×10⁶ cells per well; the mixture was then cultured overnight. After changing the medium, the cells were treated with 25 ng/ml DNP-specific IgE and sensitized by incubation for 4 h at 37 °C in a 5% CO₂ atmosphere. Cells were then washed twice with 500 μL piperase-N,N'-bis-(2-ethanesulfonic acid) (Pipes) buffer (25 mM Pipes, 119 mM NaCl, 5 mM KCl) and incubated in Pipes buffer containing 5.6 mM glucose, 1 mM CaCl₂, and 0.1% BSA for an additional 10 min at 37 °C. The cells were then exposed to the test materials for 20 min at 37 °C following treatment with 25 ng/ml of antigen DNP-BSA for 30 min at 37 °C to activate the cells. The supernatant was then transferred to a 96-well plate and incubated with substrate (1 mM p-nitrophenyl-N-acetyl-β-D-glucosaminide) for 1 h at 37 °C. To terminate the reaction, 0.1 M Na₂CO₃/NaHCO₃ or 0.2 M glycine was added to the mixture. Absorbance was then measured using an ELISA reader (SPECTRA MAX 340PC, Molecular Devices, Sunnyvale, CA) at 405 nm.

### 3. Results and discussion

#### 3.1. Structure determination of isolated compounds

Chromatographic separation of the n-hexane and EtOAc partitions of the ethanol extract from *G. pentaphylla* stems led to the isolation of 16 secondary metabolites following the performance of several steps. We determined that compounds 1–4 were new geranylated quinolone alkaloid derivatives. In addition, 12 previously described quinazoline alkaloids (5, 6, 11), an anthranilamide derivative (7), an acridone alkaloid (8), phenolic analogues (9, 10, 12–15), and a flavonoid (16) were identified when we compared their spectroscopic data to literature values, namely glycosine (5) [36], glycoliphyme (6) [37], glycomide A (7) [6], arborinine (8) [38], salicylic acid (9) [39], cis-p-coumaric acid (10) [40], glycosicine (11) [41], trans-p-coumaric acid (12) [42], protocatechueic acid (13) [43], 4-hydroxybenzoic acid (14) [44], vanillic acid (15) [45], and vitexin (16) [46]. The basic
structural characteristics of the new isolated compounds were determined via UV spectra analysis. Compounds 1–4 exhibited UV spectra that were considered typical for the 2-quinolone skeleton; their maxima values were located at ~229, ~269, ~282, and ~318 nm and shoulder maxima at ~335 nm [47].

Compound 1 was obtained as a pale brown gum. Its molecular formula was determined as C_{20}H_{25}NO_{2} by HRESIMS (m/z 312.1949, calcd for C_{20}H_{26}NO_{2} 312.1958 \[\text{M} + \text{H}\]^{+}), requiring nine degrees of unsaturation. The IR spectra displayed the presence of methyl and methylene groups (2919 and 2854 cm\(^{-1}\)), \(\alpha,\beta\)-unsaturated carbonyl (1707 and 1629 cm\(^{-1}\)), and aromatic (1576, 1510, and 1463 cm\(^{-1}\)) functionalities. Improved bismuth potassium iodide reagent revealed the presence of an improved bismuth potassium iodide reagent showed a positive result; thus indicating 1 as an alkaloid [48].

The \(^1\)H and \(^{13}\)C NMR spectra of 1 (Table 1) displayed signals for an N-methyl group [\(\delta_{\text{H}} 3.70 (3\text{H}, \text{s}, \text{-CH}_3)\); \(\delta_{\text{C}} 29.3\)], a conjugated olefinic group [\(\delta_{\text{H}} 6.15 (1\text{H}, \text{s}, \text{-H})\); \(\delta_{\text{C}} 96.6\) (C-3), 162.3 (C-4)], a conjugated carbonyl group [\(\delta_{\text{C}} 164.3\) (C-2)], and four resonances in the aromatic region [\(\delta_{\text{H}} 8.03 (1\text{H}, \text{d}, J = 7.7\text{ Hz}, \text{-H})\), 7.26 (1H, t, \(J = 7.7\text{ Hz}, \text{-H}\)), 7.60 (1H, t, \(J = 7.7\text{ Hz, -H}\)), and 7.37 (1H, d, \(J = 7.7\text{ Hz, -H}\)); \(\delta_{\text{C}} 123.7\) (C-5), 122.0 (C-6), 131.3 (C-7), and 114.2 (C-8)]. These results suggest the presence of an ortho-disubstituted phenyl group that corresponds to an N-methyl-quinolone skeleton [49]. The aforementioned 1D-NMR data of 1 were similar to those of 4-O-geranyl-quinolin-2-one from previous synthetic studies, except that an N-methyl group of tertiary amine was present in 1 instead of a secondary amine group [50,51]. To elucidate its partial structure, heteronuclear multiple bond correlation (HMBC) and \(^1\)H–\(^1\)H correlated spectroscopy (COSY) experiments were performed. The HMBC spectrum showed long-range couplings such as H-3 to C-2, C-4, and C-10; H-5 to C-4, C-7, and C-9; H-6 to C-4, C-7, C-8, and C-10; H-7 to C-5, C-8, and C-9; H-8 to C-6 and C-10; and N-methyl to C-2 and C-9. These HMBC correlations, together with the corresponding \(\Delta^Z\) disubstituted olefinic bond was also determined as \(\Delta^Z\) configuration) [53]. Based on the above spectroscopic evidence, the structure of 1 was deduced as 4-O-geranyl-N-methyl-2(1H)-quinoline and named glycopentanol A.

The molecular formula of compound 2 was C_{20}H_{25}NO_{4}, based on its HRESIMS at m/z 344.1853 (calcd for C_{20}H_{26}NO_{4} m/z 344.1856 \[\text{M} + \text{H}\]^{+}). The 1D NMR spectroscopic data (Table 1) analysis suggested that 2 shares an identical N-methyl-2(1H)-quinoline skeleton with 1, except for the presence of a modified geranylated group. The signals for the geranyl group were replaced by resonances for a 7-hydroperoxy-3,7-dimethyl-2,5-octadienyl moiety [\(\delta_{\text{H}} 5.69 (1\text{H}, \text{dd}, J = 7.7\text{ Hz, -H})\), 5.55 (1H, d, \(J = 6.3\text{ Hz, -H}\)), 4.71 (2H, d, \(J = 6.3\text{ Hz, -H}\)); 2.82 (2H, m, H-4′), 1.76 (3H, s, H-10′), and 1.35 (6H, s, H-8′, 9′); \(\delta_{\text{C}} 140.7\) (C-3′), 136.2 (C-6′), 127.9 (C-5′), 119.5 (C-2′), 82.0 (C-7′), 65.6 (C-1′), 42.1 (C-4′), 24.4 (C-8′, 9′), and 16.9 (C-10′)]. In addition, the spectral data compared well with those of the \((2E,5E)-7\text{-hydroperoxy-3,7-dimethyl-2,5-octadienyl-O-(\text{a-\text{	extasciitilde}rhamnopyranosyl})(1\text{‴→3′})(4″\text{-trans-p-coumaroyl})-\text{\textbeta\text{-glucopyranoside}}\) previously isolated from Ligustrum robustum [54]. The unusual downfield shift of C-7′ also indicated the presence of a hydroperoxy group attached to this carbon atom [55]. HMBC correlations from \(\delta_{\text{H}} 4.71\) (H-1′) to \(\delta_{\text{C}} 161.7\) (C-4) confirmed that the substituent was attached to C-4 in the N-(1H)-quinoline skeleton (Fig. 2). The geometry of the \(\Delta^Z\) disubstituted olefinic bond was also determined as \(\Delta^Z\) based on the vicinal 1H coupling constant \(\langle J_{\text{H, -H}} \rangle = 16.1\text{ Hz}\). As a result, we determined that 2 was \(4-O-(\text{\text2E,5E}-7\text{-hydroperoxy-3,7-dimethyl-2,5-octadienyl})\text{-N-methyl-2(1H)-quinoline}\) and was thus named glycopentanol B.

Compound 3 appeared as a pale brown gum substance. HRESIMS analysis revealed the presence of [M+H]^+ at m/z 328.1902 (calcd for C_{20}H_{26}NO_{3} m/z 328.1907); thus, we determined that the molecular formula was C_{20}H_{25}NO_{3}. The NMR signals supported the presence of an N-methyl(2(1H)-quinoline skeleton substituted by a 10-carbon chain (Table 1). Three signals exhibited chemical shift values that were deemed unusual for a modified geranylated derivative. In addition, the
Table 2
Inhibitory effects of isolated compounds on antigen-induced release of β-hexosaminidase from RBL-2H3 cells.

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<th>Compounds</th>
<th>IC50 Value (μM)</th>
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<th>IC50 Value (μM)</th>
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* Compounds 3 and 4 could not be tested due to limited amounts.
* IC50 presents the concentration of a drug that is required for 50% inhibition.

observation of a CH₂ unit with chemical shifts at δC 111.3 (C-8′) and δH 4.96 (1H, brs, Ha-8′) and 4.86 (1H, brs, Hb-8′), together with the quaternary carbon δC 147.4, suggested the presence of an sp² methylene group (C-8′) linked to C-7′. Diagnostic HMBC correlations for a triplet at δH 4.10 (1H, t, J = 6.3 Hz) connected to an oxygenated carbon (δC 75.4) with C-4′ (δC 35.5), C-5′ (δC 32.7), C-7′ (δC 147.4), C-8′ (δC 111.3), and C-9′ (δC 17.6) demonstrated that this proton was H-6′.

These data confirmed that the geranyl chain was also modified by C-6′ oxidation. From the HMBC spectrum, the methylene proton signal (δH 4.67) showed a correlation with the oxygenated olefin quaternary carbon signal (δC 161.8), suggesting that the modified geranyl chain resides at C-4. We determined that the structure of 3 was the new 4-O-((2E)-6-hydroxy-3,7-dimethyl-2,7-octadienyl)-N-methyl-2(1H)-quinoline, and this compound was named glycopentanolone C.

Compound 4 was isolated as a pale brown gum. Based on the molecular ion peak [M + H]+ at m/z 344.1831 (calcd for C20H26NO4 m/z 344.1856) in the HRESIMS, we determined that the molecular formula was C20H25NO4; the m/z value was 16 amu higher than that of 3, which indicates an additional hydroxy group in 4. The NMR spectroscopic data for 4 (Table 1) were superimposable with those of 3; however, one exception was the 6-hydroxy-3,7-dimethyl-2,7-octadienyl group of 3 being replaced by a 6-hydroperoxy-3,7-dimethyl-2,7-octadienyl group in 4. The only difference between 3 and 4 was a hydroperoxy group present in 4 while 3 had a hydroxyl group. Evidence for the presence of a hydroperoxy group was the signals of H-6′ and C-6′ positioned at a lower field (δH 4.08, t, J = 6.3 Hz) and δC 75.4 in 3; δH 4.32, t, J = 6.3 Hz) and δC 88.7 in 4). Therefore, the structure of 4 was elucidated as 4-O-((2E)-6-hydroperoxy-3,7-dimethyl-2,7-octadienyl)-N-methyl-2(1H)-quinoline and was given the trivial name glycopentanolone D. Owing to the presence of low amounts of 3 and 4, the C-6′ configuration could not be defined.

3.2. Inhibitory effects of isolated compounds on β-hexosaminidase release from RBL-2H3 cells

To identify compounds that exhibited inhibitory effects on mast cell degranulation, we performed a bioassay-guided separation and evaluated the inhibitory effects of the isolated compounds on β-hexosaminidase release from RBL-2H3 cells. Cells were incubated overnight in 24-well cluster plates with DNP-specific IgE. Prior to stimulation with DNP-BSA, medium change to PIPES buffer containing indicated concentration of the isolated compounds was performed and the release of β-hexosaminidase measured. Table 2 shows the inhibitory effects of the secondary metabolites isolated from the stems of G. pentaphylla during antigen-induced β-hexosaminidase release from RBL-2H3 cells. Compounds 1, 2, 5, 8–14, and 16 inhibited the release of β-hexosaminidase, with IC50 values between 0.05 and 85.70 μM, when compared to the positive control ketotifen at 31.49 μM. Ketotifen, an anti-allergic drug, possesses anti-histaminic and anti-anaphylactic properties [56,57]. Among these, compounds 1, 5, 8, and 11 were the most active alkaloids, with IC50 values between 0.05 and 4.28 μM. Compounds 12 and 14 also exhibited prominent anti-allergic activities, with IC50 values of 22.89 and 30.70 μM, respectively. In addition, 2, 9, 10, 13, and 16 displayed marginal activities with IC50 values ranging from 47.93 to 85.68 μM. When we analyzed the bioassay results and the structural characteristics of the isolated compounds, evident structure-activity relationships could not be determined. Cell viability measured by the MTT assay showed that the tested compounds did not exhibit any significant cytotoxicity to the RBL-2H3 cells at concentrations that inhibited β-hexosaminidase release (data not shown).

4. Conclusions

A chemical investigation conducted using G. pentaphylla to obtain new constituents affecting β-hexosaminidase release led to the isolation and identification of 16 compounds including four new geranylated 2(1H)-quinolone alkaloids. The structures of these compounds were identified using extensive spectroscopic methods including 2D-NMR experiments such as £H–1H COSY, HSQC, and HMBC. Some geranylated 2-quinolone alkaloid derivatives investigated were either previously isolated from natural sources or obtained synthetically [50,58]. The isolated compounds were tested to determine their inhibitory effects on the release of β-hexosaminidase from RBL-2H3 cells. The results revealed that compounds 1, 5, 8, and 11, which are alkaloids with an N-methyl moiety, exhibited potent inhibitory activities. The current study supports the medicinal use of the stems of G. pentaphylla in the treatment of type I allergy. Further studies are however warranted to elucidate the mechanism by which these active compounds inhibit the release of β-hexosaminidase.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bios.2019.03.069.

References
