Bioactive Phenylethanoids and Coumarines from *Basalmocitrus cameroonensis*

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Two new phenylethanoids, basalethanoid A (**1**) and B (**2**), and one new ceramide, basalamide A (**3**), together with eleven known compounds (**4** – **14**) were isolated from the MeOH extract of the stem barks of *Basalmocitrus cameroonensis*. The structures of all compounds were determined by comprehensive analyses of their 1D and 2D NMR, mass spectral (EI and ESI) data, chemical reactions, and comparison with previously known analogs. Compounds **1**, **2**, **5**, and **7** – **10** demonstrated a strong inhibition on reactive oxygen species (ROS) production in the oxidative burst activity of whole blood on activation with serum opsonized zymosan in the range of $IC_{50} = 0.06 - 12.30\ \mu g\ mL^{-1}$.

Key words: Basalmocitrus cameroonensis, Rutaceae, Phenylethanoid, Ceramide, Oxidative Burst Inhibition

Many clinical disorders are associated with the immune system. Suppression of the immune system is required in the management and treatment of inflammation and allergic diseases, while stimulation is highly desirable for the treatment of HIV, immunodeficiency and infectious diseases [1]. Immune suppression and cytotoxic activity affecting the function of the immune system have been reported for many synthetic and natural agents [2]. Various disease conditions such as infections, organ transplantation, cancer, rheumatoid arthritis, and systemic lupus erythromatosus are currently treated with novel immunomodulating agents [3].

In continuation of our research for bioactive molecules from Cameroonian rainforest medicinal plants, *Basalmocitrus cameroonensis* (Rutaceae), a shrub or small tree with young thorny seedlings, was studied. This plant is used as food and as remedy for several diseases in Cameroon. The seeds of *B. cameroonensis* are prepared to obtain oil and the adhesive [4]. To the best of our knowledge, no phytochemical investigation has been reported on this genus. This paper reports the isolation and structure elucidation of two new phenylethanoids (**1** and **2**) and one new ceramide (**3**) (Fig. 1), and eleven known compounds (**4** – **14**) from *B. cameroonensis*, together with data on the immunomodulatory activity of the isolated compounds.

Results and Discussion

The air-dried stem bark of *B. cameroonensis* was powdered and extracted with MeOH. The crude extract was separated by repeated column chromatography and preparative TLC (PTLC) to afford the two new phenylethanoids **1** and **2**, one new ceramide (**3**), and eleven known compounds identified as (2*S*,3*S*,4*R*,5*R*,14*E*)-1,3,4,5-tetrahydroxy-2-[(2′*R*)-2′-hydroxy octadecanoylamino]tetracos-14-ene (**4**), (*R*)-byakangelicin (**5**), *trans*-fagaramide (**6**), 4,5- dimethoxysalicylaldehyde (**7**), marmesin (**8**), scopoletin (**9**), marmin (**10**), stigmasterol-3-*O*-β-D-

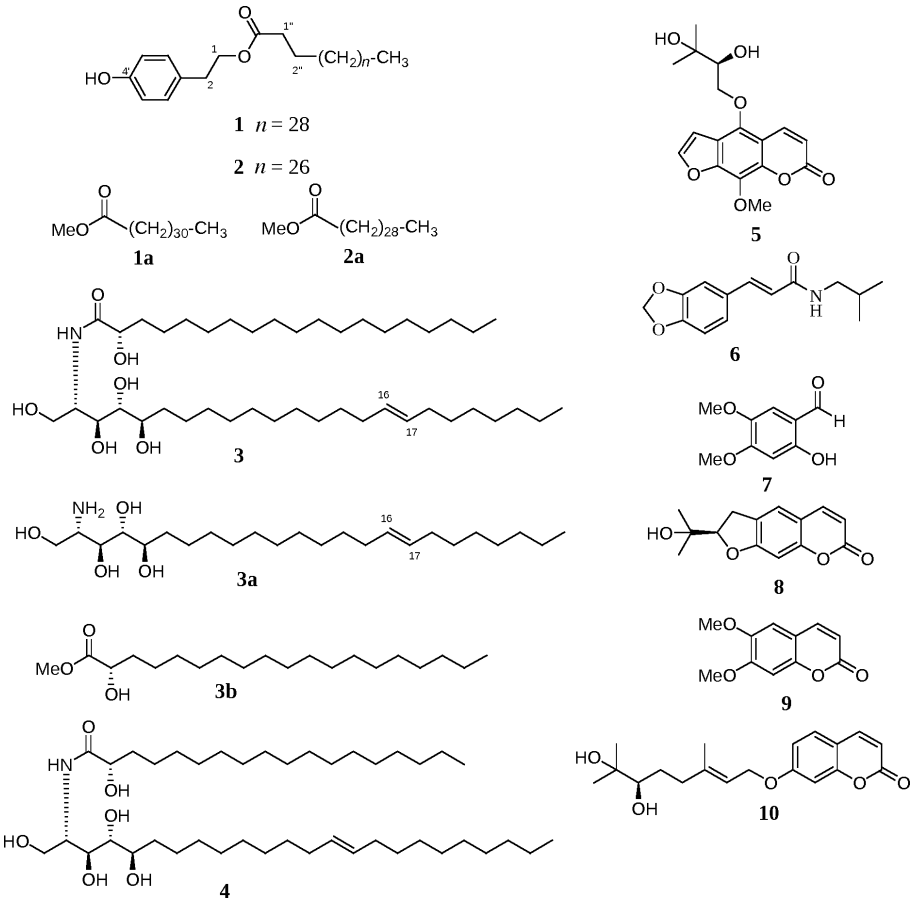

Fig. 1. Structures of compounds isolated from *B. cameroonensis*.

glucopyranoside, stigmasterol, betulinic acid, and lupeol [5 – 10].

Basalethanoid A (**1**) and B (**2**) were obtained as white amorphous powders, showing a positive reaction with $FeCl_3$ indicating their phenolic nature. Their UV spectrum exhibited two absorption maxima at 279 and 235 nm characteristic of phenylethanoids [11, 12]. The presence of hydroxyl and ester functions was indicated by two IR bands at 2915 and 1730 cm^{-1}, respectively. From the R-ESI-MS, the molecular composition was found to be $C_{40}H_{72}O_3Na$ by $[M+Na]^+$ at m/z = 623.5380 (calcd. 623.5385) and $C_{38}H_{68}O_3Na$ by $[M+Na]^+$ at m/z = 595.5070 (calcd. 595.5072), respectively.

The ^1H NMR spectra of **1** and **2** showed the typical AA′XX′ system of a *p*-disubstituted benzene ring at δ = 7.04 (d, J = 8.1 Hz, H-2′, H-6′) and 6.79 (d, J = 8.1 Hz, H-3′, H-5′), the presence of a CH_2CH_2O unit at δ = 4.22 (t, J = 6.9 Hz, H-1), and 2.84 (t, J = 6.9 Hz, H-2), and a free hydroxyl group at δ = 8.53 (br s, OH-4′) exchangeable with D_2O. This inference was supported by the ^{13}C NMR and DEPT data, which showed characteristic signals of a *p*-disubstituted benzene ring at δ = 115.4 (C-3′, C-5′), 128.2 (C-1′), 129.7 (C-2′, C-6′) and 155.7 (C-4′), and the CH_2CH_2O unit at δ = 65.0 (C-1) and 34.2 (C-2) [13]. Furthermore, in the ^1H NMR spectrum, a terminal methyl at δ = 0.89 (t, J = 6.9 Hz) and methylenes at δ = 2.29 (t, J = 7.5 Hz, -CH_2-CO-), 1.58 (m, CH_2-CH_2-CH_2-CO-), and 1.25 (br s, *n*H) were also observed. These data suggested the presence of a long chain linked to a 4-hydroxyphenylethanol moiety. The presence of a long chain was further confirmed by the ^{13}C NMR spectrum, which showed characteristic signals at δ = 173.7 (C-1″), 34.1 (C-2″), 31.8 – 29.3 $(CH_2)_n$, 24.8 (C-3″), and 14.0 (CH_3) [14].

To determine the linkage between the long chain and the 4-hydroxyphenylethanol moiety, an HMBC experiment was used. In the HMBC spectrum, correlation of H-1 (δ = 4.22) with C-1″ (δ = 173.7), C-1′ (δ = 128.2)

and C-2 (δ = 34.2) suggested that the long chain is linked to the 4-hydroxyphenylethanol moiety by an ester function.

The methanolysis of **1** and **2** yielded methyl dotriacontanoate (**1a**) (identified by ESI-MS which indicated a pseudomolecular ion at *m/z* = 517 $[M+Na]^+$, corresponding to a molecular formula $C_{33}H_{66}O_2$), methyl triacontanoate (**2a**) (identified by ESI-MS which indicated a pseudomolecular ion at *m/z* = 489 $[M+Na]^+$, corresponding to a molecular formula $C_{31}H_{62}O_2$) and 4-hydroxyphenylethanol. The latter was identified by ^{1}H NMR and EI-MS. From these spectroscopic data, basalethanoid A (**1**) and B (**2**) were characterized as 2(4-hydroxyphenethyl)dotriacontanoate and 2(4-hydroxyphenethyl)hentriacontanoate, respectively. Phenylethanoids have previously been described in the genus *Citrus* and *Fagara* (Rutaceae) [11, 12].

Basalamide A (**3**) was obtained as a brown amorphous powder. The IR spectrum showed an absorption band at 3480 cm^{-1} due to the OH functions, a strong absorption band at 1665 cm^{-1} indicating the presence of a secondary amide group [15], and at 2950, 2900 and 1505 cm^{-1} (aliphatic) suggesting it to be a fatty acid amide. The molecular composition was found to be $C_{43}H_{85}NO_6Na$ by ESI-MS (*m/z* = 737, $[M+Na+2H]^+$) and HR-ESI-MS ($[M+Na]^+$ at *m/z* = 734.6270, calcd. 734.6275). The EI-MS of **3** exhibited a peak at *m/z* = 693 due to the loss of a water molecule from the molecular ion.

The ^{1}H NMR spectrum of **3** displayed a downfield doublet at δ = 7.37 (d, *J* = 9.4 Hz, NH), a very strong aliphatic methylene band at δ = 1.26 – 1.46, as well as the signals of six protons at δ = 0.87 (t, *J* = 6.9 Hz, H-19′ and H-24) and five free hydroxyl groups at δ = 3.98, 4.24, 4.42, 4.47 and 4.50, all exchangeable with D_2O. The ^{13}C NMR and DEPT spectral data of **3** were supportive of the above analysis, showing a carbonyl group at δ = 172.2 (C-1′), one double bond at δ = 130.1 (C-16 or C-17) and 130.7 (C-16 or C-17), oxygenated carbons at δ = 75.7 (C-3), 75.1 (C-2′), 72.4 (C-5), 71.5 (C-4), 60.9 (C-1), and 51.9 (C-2), aliphatic methylenes at δ = 22.5 – 32.8, and two methyls at δ = 14.3 (C-19′ and C-24) [16, 17]. The correlations of δ = 7.37 (NH) with δ = 172.2 (C-1′), 75.7 (C-3) and 51.9 (C-2) observed in the HMBC spectrum (Fig. 2), which confirmed the presence of an amide function in compound **3**, suggested it to be a ceramide [18]. The chemical shifts of the allylic methylene carbons in **3** were assigned at δ = 32.5 (C-15 or C-18) and δ = 32.4 (C-15 or C-18) based on the clearly observed HMBC

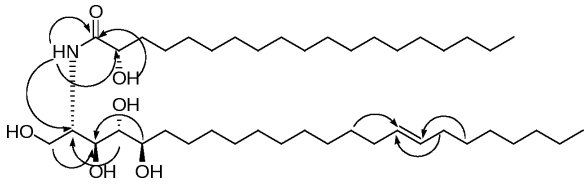

Fig. 2. Selected HMBC correlations for compound **3**.

correlations with the olefinic signals at δ = 5.37 (m, H-16 and H-17). Since the chemical shifts of allylic methylene carbons are different when alkene double bonds are *cis*-oriented ($\delta < 27$ ppm) and *trans*-oriented ($\delta > 30$ ppm) [19], the double bond in **3** was assigned an *E*-configuration.

In order to determine the lengths of the sphingosine and fatty acid chains, the position of the double bond, and the absolute configuration of **3**, the acid methanolysis method of Gaver and Sweeley was used [18]. A sphingosine (**3a**) and a fatty acid methyl ester (**3b**) were obtained by methanolysis of compound **3**. The ^{1}H NMR data of **3a** and **3b** indicated that the double bond was located in the sphingosine unit in **3**. The length of the fatty acid chain **3b** was determined by EI-MS, which showed significant fragment ion peaks at *m/z* = 297 $[CH_3(CH_2)_{16}CHOHCO]^+$, 312 $[CH_3(CH_2)_{16}CHOHCONH]^+$, and 356 $[CH_3(CH_2)_{16}CHOHCONHCH_2CH_2OH]^+$. The length of sphingosine **3a** was determined by the characteristic ions at *m/z* = 355 $[CH_3(CH_2)_6CH{=}CH(CH_2)_{10}(CHOH)_3]^+$, 325 $[CH_3(CH_2)_6CH{=}CH(CH_2)_{10}(CHOH)_2]^+$ and by the base peak *m/z* = 278 $[CH_3(CH_2)_6CH{=}CH(CH_2)_{10}CH]^+$ in the EI-MS [20]. The peak at *m/z* = 278 $[CH_3(CH_2)_6CH{=}CH(CH_2)_{10}CH]^+$ and the typical fragment ion at *m/z* = 472 $[CH_3(CH_2)_6CH{=}CH(CH_2)_{10}(CHOH)_3CH(CH_2OH)NHCOH{=}COH]^+$ formed by elimination of heptadecene from the molecular ion at *m/z* = 711 through McLafferty rearrangement confirmed that the double bond is on the long chain base. In order to determine the position of the double bond, MS-MS fragmentations of **3a** were studied, and the results indicated the double bond to be between C-16 and C-17 (Fig. 3). Cross peaks in the ^{1}H-^{1}H COSY spectrum were observed between an amide proton (δ = 7.37) and H-2 (δ = 4.08), which, in turn, was coupled to three protons at δ = 3.57 (H-1a), δ = 3.55 (H-1b), and δ = 3.38 (H-3). Furthermore, H-3 showed correlations with H-2 and with H-4, and finally H-4 showed correlation with H-5 (δ = 3.38). No cross peaks were observed of the signal at δ = 3.97 (H-2′)

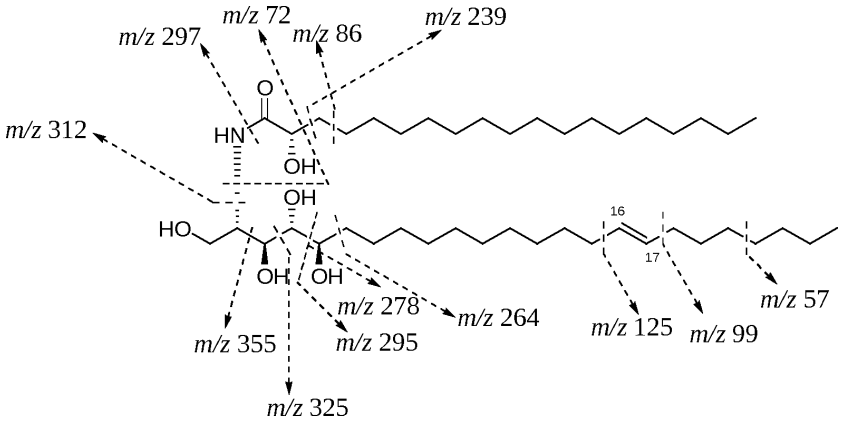

Fig. 3. Selected mass fragmentation patterns for compound **3**.

to any downfield proton signals, but in the HMBC spectrum it showed strong correlation with C-1′ (δ = 172.2). This suggested that the fifth hydroxyl group is present at C-2′ of the fatty acid chain. The positions of the four hydroxyl groups in the long chain base were further confirmed by the mass fragmentation pattern (Fig. 3) as well as by the HMBC correlations. Thus the long chain base and fatty acid of **3** must be (*E*) 2-aminotetracos-16-ene-1,3,4,5-tetraol and 2-hydroxynonadecanoic acid, respectively. On the basis of this evidence, the structure of basalamide A (**3**) was determined to be 2-hydroxy-*N*-((*E*)-1,3,4,5-tetrahydroxytetracos-16-en-2-yl)nonadecanamide.

The stereochemistry at the chiral centers C-1 to C-4 has already been established in nubenamide [19]. By comparison of the optical rotation value of **3a** $\{[\alpha]_D^{28} = +8.4$ (c = 0.20, MeOH)$\}$ with the literature value of nubenamide, the absolute configuration of basalamide A (**3**) was found to be 2*S*, 3*S*, 4*R*, and 5*R*. In the same manner, the optical rotation value of **3b** $[\alpha]_D^{28} = +22.8$ (c = 0.60, $CHCl_3$) suggested the absolute configuration of C-2′ of the fatty acid to be *R* by comparison with the literature values of tithoniamide B [21]. Thus, the structure of compound **3** was assigned as (2*S*,3*S*,4*R*,5*R*,16*E*)-1,3,4,5-tetrahydroxy-2-[(2′*R*)-2′-hydroxynonadecanoylamino]tetracos-16-ene.

Ceramides, which are mostly found in cell walls of plants and animals, have already been reported in the literature [16, 18 – 21].

Compounds **1**, **2**, **5**, and **7 – 10** were screened over a wide range of concentrations (3.1 – 50 μg mL^{-1}) for their immunomodulatory potential. These compounds were shown to possess inhibitory activity upon activation with serum opsonized zymosan, which was tested *in vitro* for oxidative burst studies of whole blood. Compounds **7 – 14** showed significant effects on the oxidative burst of the whole blood (IC_{50} range 0.06 – 0.09 μg mL^{-1}), while compounds **1**, **2**, and **5** showed inhibition activity (IC_{50} range 7.00 – 12.30 μg mL^{-1}) equal to the control (Ibuprofen IC_{50} =11.20 μg mL^{-1}) (Table 1). The test compounds exhibited a clear suppressive effect on phagocyte oxidative burst response upon activation with serum opsonized zymosan in a dose-dependent manner.

Table 1. Effect of compounds **1**, **2**, **5** and **7 – 10** on oxidative burst of whole blood.

Compounds	IC_{50} (μg mL^{-1})	Compounds	IC_{50} (μg mL^{-1})
1	11.70 ± 2.20	**2**	12.30 ± 3.50
5	7.00 ± 1.40	**7**	0.09 ± 0.02
8	0.08 ± 0.01	**9**	0.06 ± 0.01
10	0.07 ± 0.01	Ibuprofen	11.20 ± 1.90

Experimental Section

General

Optical rotations $[\alpha]_D$(MeOH or $CHCl_3$, c in g mL^{-1}) were determined by using a JASCO digital polarimeter (model DIP-3600). Infrared spectra were recorded on a JASCO FT/IR-410 spectrophotometer. UV spectra were determined on a Spectronic Unicam spectrophotometer. HR-ESI-MS were recorded on an APEX III (Bruker Daltonik) 7 Tesla (ESI-FT-ICR-MS). EI-MS spectra were recorded on a Finnigan MAT 95 spectrometer (70 eV) with perfluorkerosene as reference substance for HR-EI-MS. The ^{1}H and ^{13}C NMR spectra were recorded at 500 and 125 MHz, respectively, on a Bruker AMX 500 NMR spectrometer. Methyl, methylene and methine carbons were distinguished by DEPT experiments. Homonuclear ^{1}H connectivities were determined by using the COSY experiment. One-bond ^{1}H-^{13}C connectivities

were determined with HMQC gradient pulse factor selection. Two- and three-bond ^{1}H-^{13}C connectivities were determined by HMBC experiments. Chemical shifts are reported in δ (ppm) using TMS as internal standard and coupling constants (J) are measured in Hz. Column chromatography was carried out on silica gel (70 – 230 mesh, Merck) and flash silica gel (230 – 400 mesh, Merck). TLC was performed on Merck precoated silica gel 60 (F_{254}, Merck), and spots were visualized by using ceric sulfate spray reagent. All reagents used were of analytical grade.

Collection and identification

The stem barks of *B. cameroonensis* was collected and identified by Mr. Nana Victor of the National Herbarium, Cameroon, in April 2006 at Batouri locality, Eastern Cameroon. The herbarium specimen documenting the collection has been deposited in the National Herbarium, Yaoundé, Cameroon (Ref. No. 6159 SRF/CAM).

Extraction and isolation

Air-dried, powdered stem barks of *B. cameroonensis* (5.5 kg) were extracted with MeOH at r. t. for 72 h. After removing the solvents by evaporation under reduced pressure, the obtained crude extract (165.0 g) was chromatographed over silica gel 60 (230 – 400 mesh), using hexane, ethyl acetate and MeOH in increasing polarity order. A total of 123 sub-fractions (*ca.* 250 mL each) were collected and combined on the basis of TLC analysis leading to four main fractions A – D.

Fraction A (22.0 g) consisted of the combined sub-fractions 1 – 15 eluted with a mixture of hexane-ethyl acetate (9 : 1). Fraction B (13.0 g) was constituted of the sub-fractions 16 – 45 eluted with a mixture of hexane-ethyl acetate (7 : 3), and main fraction C (55.0 g) of sub-fractions 46 – 85 eluted with hexane-ethyl acetate (1 : 1). Fraction D (25.0 g) was constituted of sub-fractions 86 – 123 eluted with ethyl acetate and with a mixture of ethyl acetate-MeOH (9 : 1).

Fraction A was chromatographed over a silica gel 60 column with a hexane-CH_2Cl_2 gradient. A total of 33 fractions of *ca.* 100 mL each were collected and combined on the basis of TLC. Fractions 1 – 20 yielded lupeol (155.5 mg), fractions 21 – 33 yielded a mixture of sterols (stigmasterol + sitosterol) (30.0 mg) and fatty acids. Fraction B was chromatographed over a silica gel 60 column with a hexane-CH_2Cl_2 gradient. A total of 20 fractions of *ca.* 100 mL each were collected and combined on the basis of TLC. Fractions 1 – 15 were further chromatographed over silica gel 60 with the mixture of hexane-CH_2Cl_2 (3 : 1) to yield *trans*-fagaramide (**6**) (10.5 mg), scopoletin (**9**) (40.0 mg), 2(4-hydroxyphenethyl)dotriacontanoate (**1**) (13.4 mg) and 2(4-hydroxyphenethyl)hentriacontanoate (**2**) (19.1 mg). Fraction C was chromatographed over a silica gel 60 column with a hexane-CH_2Cl_2-MeOH gradient. A total of 50 fractions of *ca.* 100 mL each were collected and combined on the basis of TLC. Fractions 5 – 30 were further chromatographed over silica gel 60 with a mixture of hexane-CH_2Cl_2 (1 : 4) to yield 4,5-dimethoxysalicylaldehyde (**7**) (15 mg), marmin (**10**) (125.1 mg), marmesin (**8**) (69.0 mg), betulinic acid (15.2 mg) and (*R*)-byakangelicin (**5**) (18.0 mg). Fraction D was chromatographed over a silica gel 60 column with a CH_2Cl_2-MeOH (2 : 1) gradient. A total of 35 fractions of 100 mL each were collected and combined on the basis of TLC. Fractions 1 – 12 yielded the β-D-glucopyranoside of stigmasterol (15.0 mg) and (2*S*,3*S*,4*R*,5*R*,16*E*)-1,3,4,5-tetrahydroxy-2-[(2′*R*)-2′-hydroxynonadecanoylamino]tetracos-16-ene (**3**) (18.9 mg). Fractions 18 – 20 yielded (2*S*,3*S*,4*R*,5*R*,14*E*)-1,3,-4,5-tetrahydroxy-2-[(2′*R*)-2′-hydroxy octadecanoylamino]-tetracos-14-ene (**4**) (25.0 mg).

Chemical derivatives

*Methanolysis of compounds **1** and **2***

Compounds **1** and **2** (5.0 mg each) were added to a mixture of HCl (3.5 mL, 1 N) and dry MeOH (6.0 mg) and refluxed for 16 h with magnetic stirring. Then H_2O (10.0 mL) was added to the refluxed mixture, which was extracted with *n*-hexane (3 × 10 mL). The fatty acid methyl esters (**1a**, 2.0 mg and **2a** 1.4 mg) were obtained after the purification of the *n*-hexane extract over a silica gel column with *n*-hexane-CH_2Cl_2 (9 : 1) as solvent.

*Methanolysis of compound **3***

Compound **3** (7.5 mg), was added to a mixture of HCl (3.5 mL, 1N) and dry MeOH (6.0 mg), and refluxed for 16 h with magnetic stirring. Then H_2O (10.0 mL) was added to the refluxed mixture, which was extracted with *n*-hexane (3 × 10 mL). The fatty acid methyl ester (**3b**, 2.0 mg) was obtained after the purification of the *n*-hexane extract over a silica gel column with *n*-hexane-CH_2Cl_2 (9 : 1) as solvent. The MeOH/H_2O phase was evaporated under reduced pressure. The residue obtained was also purified over a silica gel column and eluted with CH_2Cl_2-MeOH (15 : 1) to yield a sphingosine (**3a**, 1.5 mg).

*2(4-Hydroxyphenethyl)dotriacontanoate (**1**)*

White amorphous powder. – UV (MeOH): λ_{max} (log ε) = 279 (3.8), 235 (3.9) nm. – IR ($CHCl_3$): ν_{max} = 2915, 2850, 1730, 1614, 1392 cm^{-1}. – ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.89 (t, J = 6.9 Hz, 3H, 31″-H), 1.25 (br s, 56H, 4″-30″-H), 1.58 (m, 2H, 3″-H), 2.29 (t, J = 7.5 Hz, 2H, 2″-H), 2.84 (t, J = 6.9 Hz, 2H, 2-H), 4.22 (t, J = 6.9 Hz, 2H, 1-H), 6.79 (d, J = 8.1 Hz, 2H, 3′-H and 5′-H), 7.04 (d, J = 8.1 Hz, 2H,

2′-H and 6′-H), 8.53 (br s, 1H, OH). – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 14.0 (C-19′), 24.8 (C-3″), 29.3 (methylenes), 34.1 (C-2″), 34.2 (C-2), 65.0 (C-1), 115.4 (C-3′ and C-5′), 128.2 (C-1′), 129.7 (C-2′ and C-6′), 155.7 (C-4′), 173.7 (C-1″). – MS ((+)-ESI): *m/z* = 623 $[M+Na]^+$. – HRMS ((+)-ESI): *m/z* = 623.5380 (calcd. 623.5385 for $C_{40}H_{72}O_3Na$, $[M+Na]^+$).

*Methyl dotriacontanoate (**1a**)*

White powder. – MS (EI, 70 eV): *m/z* = 494. – ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.73 (t, *J* = 7.5 Hz, 3H, -CH_3), 1.40 – 1.09 (br s, -$(CH_2)_n$-), 2.06 (t, *J* = 7.8 Hz, 2H, 2-H), 3.80 (s, 3H, -OCH_3).

*2(4-Hydroxyphenethyl)hentriacontanoate (**2**)*

White amorphous powder. – UV (MeOH): λ_{max} (log ε) = 280 (3.5), 245 (3.7) nm. – IR ($CHCl_3$): ν_{max} = 2915, 2848, 2359, 1732, 1467 cm^{-1}. – ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.89 (t, *J* = 6.9 Hz, 3H, 29″-H), 1.24 (br s, 52H, 4″-28″-H), 1.59 (m, 2H, 3″-H), 2.30 (t, *J* = 7.5 Hz, 2H, 2″-H), 2.84 (t, *J* = 6.9 Hz, 2H, 2-H), 4.22 (t, *J* = 6.9 Hz, 2H, 1-H), 6.79 (d, *J* = 8.1 Hz, 2H, 3′-H and 5′-H), 7.04 (d, *J* = 8.1 Hz, 2H, 2′-H and 6′-H), 8.53 (br s, 1H, -OH). – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 14.1 (C-19′), 24.8 (C-3″), 29.3 (methylenes), 34.1 (C-2″), 34.2 (C-2), 65.0 (C-1), 115.4 (C-3′ and C-5′), 128.2 (C-1′), 129.7 (C-2′ and C-6′), 155.7 (C-4′), 173.7 (C-1″). – MS ((+)-ESI): *m/z* = 595 $[M+Na]^+$. – HRMS ((+)-ESI): *m/z* = 595.5070 (calcd. 595.5072 for $C_{38}H_{68}O_3Na$, $[M+Na]^+$).

*Methyl triacontanoate (**2a**)*

White powder. – MS (EI, 70 eV): *m/z* = 466. – ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.66 (t, *J* = 7.4 Hz, 3H, -CH_3), 1.38 – 1.10 (br s, -$(CH_2)_n$-), 2.08 (t, *J* = 7.7 Hz, 2H, 2-H), 3.83 (s, 3H, -OCH_3).

*1,3,4,5-Tetrahydroxy-2-[(2′R)-2′-hydroxynonadecanoylamino]tetracos-16-ene (**3**)*

Brown amorphous powder. – $[\alpha]_D^{28}$ = + 9.5 (*c* = 0.15, MeOH). – IR (CH_3OH): ν_{max} = 3480, 2950, 2900, 1665, 1505 cm^{-1}. – ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 0.87 (t, *J* = 6.9 Hz, 6H, 19′-H and 24-H), 1.26 (br s, 62H, 8-14-H, 20-23-H, 4′-18′-H), 1.56 (m, 2H, 3′-H), 1.94 (m, 4H, 15-H and 18-H), 3.38 (br s, 2H, 3-H and 5-H), 3.55 (dd, *J* = 6.3, 10.6 Hz, 1H, 1b-H), 3.57 (dd, *J* = 6.3, 10.6 Hz, 1H, 1a-H), 3.67 (m, 1H, 4-H), 3.97 (dd, *J* = 4.4, 8.8 Hz, 1H, 2′-H), 3.98 (br s, 1H, 2′-OH), 4.08 (m, 1H, 2-H), 4.24 (br s, 1H, 4-OH), 4.42 (br s, 1H, 5-OH), 4.47 (br s, 1H, 3-OH), 4.50 (br s, 1H, 1-OH), 5.37 (br s, 2H, 16-H and 17-H), 7.37 (d, *J* = 9.4 Hz, 1H, -NH). – ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 14.3 (C-19′ and C-24), 22.5 (C-18′ and C-23), 25.9 (C-4′), 31.2 – 29.0 (methylenes), 31.7 (C-6), 32.4 (C-18), 32.5 (C-15), 32.8 (C-3′), 51.9 (C-2), 60.9 (C-1), 71.5 (C-4), 72.4 (C-5), 75.1 (C-2′), 75.7 (C-3), 130.1 (C-16), 130.7 (C-17), 172.2 (C-1′). – MS ((+)-ESI): *m/z* = 737 $[M+Na+2H]^+$. – HRMS ((+)-ESI): *m/z* = 734.6270 (calcd. 734.6275 for $C_{43}H_{85}NO_6Na$, $[M+Na]^+$). – MS (EI, 70 eV) data are illustrated in Fig. 3.

*Sphingosine (**3a**)*

Brown oil. – $[\alpha]_D^{28}$ = + 8.4 (*c* = 0.2, MeOH). – IR (CH_3OH): ν_{max} = 3530, 3050, 2900, 1505 cm^{-1}. – ^{1}H NMR (500 MHz, MeOD): δ = 0.85 (t, *J* = 7.0 Hz, 3H, 24-H), 1.26 (methylenes), 1.94 – 2.00 (m, 4H, 15-H and 18-H), 3.40 (br s, 1H, 3-H), 3.50 (br s, 1H, 1b-H), 3.57 (br s, 1H, 1a-H), 3.70 (m, 1H, 4-H), 3.97 (br s, 1H, 5-H), 4.26 – 4.65 (br s, 4H, 4 × OH), 5.37 (br s, 2H, 16-H and 17-H).

*Fatty acid methyl ester (**3b**)*

Yellow oil. – $[\alpha]_D^{28}$ = + 22.8 (*c* = 0.6, $CHCl_3$). – ^{1}H NMR (500 MHz, $CHCl_3$): δ = 4.15 (dd, *J* = 4.0, 7.8 Hz, 1H, 2-H), 3.80 (3H, -OMe), 1.67 – 1.80 (m, 2H, 3-H), 1.27 – 1.24 (methylene), 0.86 (t, *J* = 7.0 Hz, 3H, 19-H).

Chemiluminescence assay for determination of immunomodulation activity

A luminol-enhanced chemiluminescence assay was performed, as described in [22]. In brief, whole blood (diluted 1 : 200) and neutrophils (1×10^7) suspended in Hank's balance salt solution with calcium and magnesium ($HBSS^{++}$) were incubated with 50 μL of each test compounds at concentrations of 3.1 – 50 μg mL^{-1} for 30 min. Then, 50 μL (20 mg mL^{-1}) zymosan (Sigma Chemical Co. St. Louis, MO) followed by 50 μL (7×10^5 M) luminal (G-9382 Sigma) and then $HBSS^{++}$ were added to adjust the final volume to 0.2 mL. $HBSS^{++}$ was used as a control.

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