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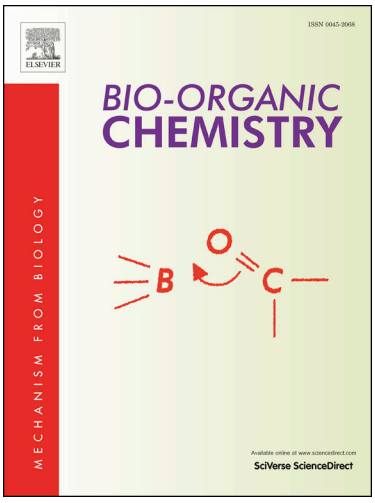

Short communication

Four new sucrose diesters of substituted truxinic acids from *Trigonostemon honbaensis* with their anoctamin-1 inhibitory activity

Ninh Khac Ban, Luu Hong Truong, Tran Van Tiep, Duong Thi Hai Yen, Vu Van Doan, Nguyen Xuan Nhiem, Yohan Seo, Wan Namkung, Seung Hyun Kim, Bui Huu Tai, Phan Van Kiem

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Four new sucrose diesters of substituted truxinic acids from *Trigonostemon honbaensis* with their anoctamin-1 inhibitory activity

Ninh Khac Ban[a,b], Luu Hong Truong[c], Tran Van Tiep[d], Duong Thi Hai Yen[a], Vu Van Doan[a], Nguyen Xuan Nhiem[a,b], Yohan Seo[e], Wan Namkung[e], Seung Hyun Kim[e], Bui Huu Tai[a,b,*], and Phan Van Kiem[a,b,*]

[a)]*Institute of Marine Biochemistry, Vietnam Academy of Science and Technology (VAST), 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam*

[b)]*Graduate University of Science and Technology, VAST, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam*

[c)]*Southern Institute of Ecology, VAST, 01 Mac Dinh Chi Street, Ho Chi Minh City, Vietnam*

[d)]*Nui Chua National Park, Ninh Thuan Province, Vietnam*

[e]*College of Pharmacy, Yonsei Institute of Pharmaceutical Science, Yonsei University, Incheon 406-840, Korea*

*Corresponding author:

Phan Van Kiem, Prof. Ph.D. E-mail: phankiem@yahoo.com

Bui Huu Tai, Ph.D. E-mail: bhtaiich@gmail.com

Institute of Marine Biochemistry, Vietnam Academy of Science and Technology

18-Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam

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Highlights

- Four new sucrose diester of substituted truxinic acids (**1**-**4**) were isolated from *Trigonostemon honbaensis*.
- Their structures were established by HR-ESI-MS, NMR, and CD spectra.
- Compounds **1**-**4** (30 μM) inhibited ANO-1 activity at levels of 27.7 ± 1.10%, 35.6 ± 0.92%, 43.7 ± 1.61%, and 40.8 ± 1.25%, respectively.

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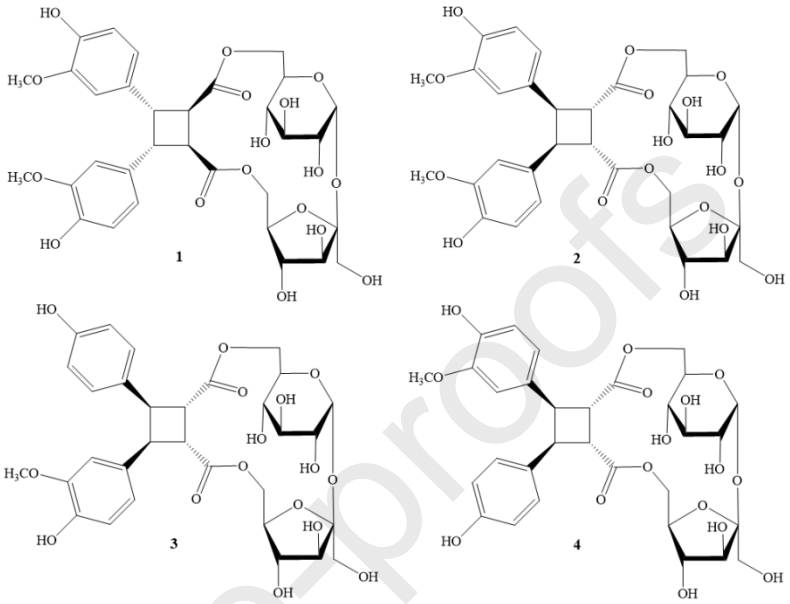
Graphical abstract

Trigonostemon honbaensis Tagane & Yahara



Truxinic acid sucrose ester analogs

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Four new sucrose diester of substituted truxinic acids from Trigonostemon honbaensis with their anoctamin-1 inhibitory activity

Ninh Khac Ban, Luu Hong Truong, Tran Van Tiep, Duong Thi Hai Yen, Vu Van Doan, Nguyen Xuan Nhiem, Yohan Seo, Wan Namkung, Seung Hyun Kim, Bui Huu Tai, and Phan Van Kiem

All authors declared no conflict of interest

On the behaft of the authors,

Corresponding author:

Phan Van Kiem, Prof. Ph.D.

Institute of Marine Biochemistry, Vietnam Academy of Science and Technology (VAST), 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam

Phone : Tel.: +84-4-3791-7053 Fax: +84-4-3791-7054 E-mail : phankiem@yahoo.com

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Abstract

Truxinic acid sucrose diesters analogs possess interesting chemical structure by the presence of cyclobutane-ring and macrocyclic sucrose diesters moieties which are rarely found from natural sources. This paper describes the isolation and structural elucidation of four new sucrose diesters of substituted truxinic acids, trigohonbanosides A-D (**1-4**), from the leaves of *Trigonostemon honbaensis*. Their chemical structures were elucidated by HR-ESI-MS, NMR, and CD spectroscopic methods. At a concentration of 30 μM, compounds **1-4** moderately inhibited ANO-1 activity with inhibitory percentages of 27.7 ± 1.10 %, 35.6 ± 0.92 %, 43.7 ± 1.61 %, and 40.8 ± 1.25%, respectively.

Keywords: *Trigonostemon honbaensis*, Trigohonbanoside, Truxinic acid sucrose diesters, Anoctamin-1 inhibitor.

Introduction

The genus *Trigonostemon* Blume (Euphorbiaceae family) consists approximately 85 species which are mainly distributed in tropical and subtropical regions of the Asia. Of these, Vietnam is considered to be the center of diversity for this genus with 22 species. Recently, the *T. honbaensis* Tagane & Yahara is recorded as a new and endemic *Trigonostemon* species, growing at Hon Ba nature reserve of Vietnam [1]. Several parts of the plants from *Trigonostemon* genus have been traditionally used as folk medicines for treatment of diarrhea, asthma, and skin diseases. Since array of interesting diterpenoids, phenolics, steroids, indole and *β*-carboline alkaloids reviewed from *Trigonostemon* genus, the chemical constituents of this plant species were intensively investigated during the last decade [2, 3]. Over 10 plants in this genus have been phytochemically

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investigated which resulted in isolation and chemical structural elucidation of over 200 compounds [3-7]. Isolated diterpenes (daphnane diterpenoid orthoesters), indole alkaloids (trigonoliimines) and *β*-carboline alkaloids (trigonoines and trigonostemines) were performed total synthesis because of their interesting chemical structure framework and/or valuable biological activities [8-10]. Above mentioned literature prompt us to carry out the phytochemical investigation of the *Trigonostemon honbaensis* species and herein, we report the isolation and structural elucidation of four new sucrose diesters of substituted truxinic acids. Anoctamin-1 (ANO-1) inhibitory activity of the isolated compounds was also evaluated using yellow fluorescent protein (YFP) reduction assay. The truxinic acid sucrose diesters analogs are very rare from natural sources. To the best of our knowledge, around five of those compounds have been isolated so far from *Imperata cylindrica*, *Coix lachryma-jobi*, *Bidens parviflora*, and oat grains [11-14].

2. Materials and Methods

2.1. General experimental procedures

Optical rotations were obtained on a Jasco P2000 polarimeter. CD spectra were recorded on a Chirascan spectrometer. HR-ESI-MS was performed on an Agilent 6530 Accurate Mass Q-TOF LC/MS system. NMR spectra were recorded on a Bruker Avance 500 MHz spectrometer. Preparative HPLC was acquired on an Agilent 1100 HPLC system equipped with quaternary pump (flow rate 3 mL/min), autosampler, DAD detector, and J'sphere ODS-H80 preparative HPLC column (20×250 mm, particle size 4 μm). Open column chromatography was performed using silica gel, reversed phase C18, sephadex LH-20, and diaion HP-20 as stationary phase. Thin layer chromatography was carried out using pre-coated silica gel 60 F_{254} and RP-18 F_{254S} plates.

2.2. Plant material

The leaves of *Trigonostemon honbaensis* Tagane & Yahara (Euphorbiaceae family) were collected at Nui Chua national park, Ninh Thuan province, Vietnam in December 2018. Its scientific name

was identified by one of the authors, Prof. Ninh Khac Ban. A voucher specimen (No. NCCT-P79) was kept at the Institute of Marine Biochemistry, Vietnam Academy of Science and Technology.

2.3. Extraction and isolation

The dried powdered leaves of *Trigonostemon honbaensis* (4 kg) were extracted three times with methanol (each 10 L) under sonication (each 60 min) at room temperature. After filtration, filtrated methanol was evaporated under reduced pressure to yield 450 g dark residue. This methanol residue was then suspended with distilled water (3.0 L) and partitioned in turn with dichloromethane and ethyl acetate to give dichloromethane (80.7 g), ethyl acetate (4.1 g), and water-soluble portions. The water layer was loaded into diaion HP-20 column, washed with water, and eluted with methanol/water (1/3, 1/1, 3/1, 1/0, v/v, each 1.5 L) to give four fractions (THW1-THW4). The fraction THW2 was chromatographed on reserved phase C18 (RP-18) column, eluting with methanol/water (1/1, v/v, 2 L) to yield six fractions (THW2A- THW2F). The fraction THW2D was then separated by sephadex LH-20 column, eluting with methanol/water (1/1, v/v, 1 L) to four fractions (THW2D1-THW2D4). Compound **1** (6.4 mg, t_R 36.6 min) was obtained by purifying fraction THW2D4 by preparative-HPLC with isocratic mobile phase of acetonitrile (20%) in water. The fraction THW2E was separated on sephadex LH-20 column, eluting with methanol/water (1/1, v/v) to yield four fractions THW2E1-THW2E4. Preparative HPLC purification of fraction W2E2 with isocratic mobile phase of acetonitrile (20%) in water gave compound **2** (4.5 mg, t_R 43.9 min). Compounds **3** (5.0 mg, t_R 43.4 min), and **4** (4.3 mg, t_R 40.8 min) were obtained from THW2E4 fraction by preparative HPLC using isocratic mobile phase of acetonitrile (20%) in water.

*2.3.1. Trigohonbanoside A (**1**)*

Light yellow amorphous powder; $[\alpha]_D^{25}$: +31.3° (*c* 0.1, MeOH); CD (MeOH) θ $mdeg_{(\lambda)}$: $-19.1_{(244)}$; HR-ESI-MS: *m/z* 729.1824 $[M+Cl]^-$ (calcd. for $C_{32}H_{38}O_{17}Cl$, 729.1798); ^1H- and ^{13}C-NMR data were shown in the Table 1.

*2.3.2. Trigohonbanoside B (**2**)*

Light yellow amorphous powder; $[\alpha]_D^{25}$: +48.1° (*c* 0.1, MeOH); CD (MeOH) $mdeg_{(\lambda)}$: $+21.5_{(245)}$; HR-ESI-MS: *m/z* 729.1798 $[M+Cl]^-$ (calcd. for $C_{32}H_{38}O_{17}Cl$, 729.1798); ^1H- and ^{13}C-NMR data were shown in the Table 1.

*2.3.3. Trigohonbanoside C (**3**)*

Light yellow amorphous powder; $[\alpha]_D^{25}$: +23.7° (*c* 0.1, MeOH); CD (MeOH) $mdeg_{(\lambda)}$: $+13.9_{(243)}$; HR-ESI-MS: *m/z* 699.1697 $[M+Cl]^-$ (calcd. for $C_{31}H_{36}O_{16}Cl$, 699.1692); ^1H- and ^{13}C-NMR data were shown in the Table 1.

*2.3.4. Trigohonbanoside D (**4**)*

Light yellow amorphous powder; $[\alpha]_D^{25}$: +26.5° (*c* 0.1, MeOH); CD (MeOH) $mdeg_{(\lambda)}$: $+18.1_{(238)}$; HR-ESI-MS: *m/z* 699.1710 $[M+Cl]^-$ (calcd. for $C_{31}H_{36}O_{16}Cl$, 699.1692); ^1H- and ^{13}C-NMR data were shown in the Table 1.

2.4. Alkaline hydrolysis

Compounds **1**-**4** were each (0.5 mg) dissolved in 1.0 mL solution of KOH (1.0 M) in methanol and heated at 60°C for 2 h. Reaction was cooled to room temperature and carefully neutralized with solution of HCl 1.0 M. The solvent was then driven out under nitrogen flow. The residue was re-dissolved in 1.0 mL of water and extracted twice with equal volume of ethyl acetate. Trace of sucrose in water layer was confirm by co-TLC analysis in comparison with authentic sucrose ($CHCl_3$: MeOH : Water = 1.0 : 1.0 : 0.15, R_f = 0.36).

2.5. Yellow fluorescent protein reduction assay

YFP reduction analysis was performed using FLUOstar Omega microplate reader and MARS Data Analysis Software. Experiments were done as previously described [15]. In brief, anoctamin-1 channel and YFP expressing Fisher Rat Thyroid (FRT) cells were plated in black-walled 96-well microplates at a density of 1.5×10^4 cells per well. Each well was washed twice (each 200 μL) with phosphate buffered saline (PBS) and then 2 μL of compound solution was added to reach final concentration of 30 μM. After incubation at 37℃ for 10 min, microplates were transferred to a plate reader for fluorescence analysis. Each well was individually measured for ANO1-mediated I^- influx by monitoring YFP fluorescence continuously (400 ms per point) for 2 s (baseline). After that, 100 μL of 140 mM I^- solution containing 200 μM ATP was added at 2 s and then YFP fluorescence was recorded for 6 s. The initial iodide influx rate was determined from the initial slope of fluorescence reduction by nonlinear regression after iodide injection with ATP.

3. Result and Discussion

Dried leaves of *T. honbaensis* were extracted with methanol and roughly separated into three polarity fractions. Low-range polarity fraction (dichloromethane extract, notably contained fatty substances, essential oil, chlorophyll) and middle-range polarity fraction (ethyl acetate extract, small amount obtained as mentioned in the Experiment) were not subjected for phytochemical investigation. Water layer were selected for purification using chromatographic methods. Chemical structures of isolated compounds were elucidated by HR-ESI-MS, NMR, and CD spectroscopic methods to reveal four new compounds **1**-**4**.

Compound **1** was obtained as light-yellow amorphous powder. The negative mode HR-ESI-MS analysis of **1** exhibited a pseudo-molecular ion peak at *m/z* 729.1824 $[M+Cl]^-$, indicating the molecular formula of $C_{32}H_{38}O_{17}$ (calcd. for $C_{32}H_{38}O_{17}Cl$, 729.1798). The ^{1}H-NMR spectrum of **1** displayed two set of ABX coupled spin systems [δ_H 6.34 (1H, d, J = 2.0 Hz); 6.60 (1H, d, J = 8.5 Hz), 6.48 (1H, dd, J = 2.0 and 8.5 Hz), 6.43 (1H, d, J = 2.0 Hz), 6.66 (1H, d, J = 8.5 Hz), 6.61 (1H,

dd, J = 2.0 and 8.5 Hz)], two methoxy groups [δ_H 3.61 and 3.63 (each, 3H, s)], and one anomeric proton [δ_H 5.37 (1H, d, J = 3.5 Hz)]. The ^{13}C-NMR spectrum of **1** showed 32 carbon signals, which were assigned thanks to HSQC spectrum into 9 non-protonated carbons, 3 methylenes, 18 methines and 2 methyl carbons. Twelve olefinic carbons (δ_C 113.1 ~ 148.7) and two methoxy carbons (δ_C 56.3 and 56.4) expected a pair of 3-methoxy-4-hydroxylphenyl groups. This deduction was confirmed by HMBC correlations between H-2 (δ_H 6.43)/ H-6 (δ_H 6.61) and C-4 (δ_C 146.1), H-5 (δ_H 6.66)/ 3-OC<u>H</u>$_3$ (δ_H 3.63) and C-3 (δ_C 148.7), H-2′ (δ_H 6.34)/ H-6′ (δ_H 6.48) and C-4′ (δ_C 146.1), H-5′ (δ_H 6.60)/ 3′-OC<u>H</u>$_3$ (δ_H 3.61) and C-3′ (δ_C 148.5). Four aliphatic methines [δ_C 46.2 (C-7), 44.8 (C-8), 45.2 (C-7′), 44.7 (C-8′)] and COSY cross peaks of H-7 (δ_H 4.20)/ H-8 (δ_H 3.88)/ H-8′ (δ_H 3.97)/ H-7′ (δ_H 4.25)/ H-7 were attributed to a cyclobutane ring (Figure 2). Moreover, HMBC correlations between H-7 (δ_H 4.20) and C-1 (δ_C 131.8)/ C-2 (δ_C 113.7)/ C-6 (δ_C 121.4), H-7′ (δ_H 4.25) and C-1′ (δ_C 131.7)/ C-2′ (δ_C 113.1)/ C-6′ (δ_C 121.6), H-8 (δ_H 3.88)/ H-8′ (δ_H 3.97) and carbonyl carbons C-9 (δ_C 174.5)/ C-9′ (δ_C 174.2) proved the presence of truxinic acid moiety. Remaining twelve oxygenated carbons (δ_C 61.9 ~ 106.8) were assigned to a disaccharide moiety. Moreover, resonant signals of an anomeric methine (δ_C 94.7/ δ_H 5.37) and non-protonated anomeric carbon (δ_C 106.8) were expected for sucrose disaccharide as previously reported in the literature [16, 17]. The presence of sucrose moiety was also suggested by the small coupling constant of anomeric proton of glucose unit [δ_H 5.37 (d, J = 3.5 Hz, Glc H-1″), HMBC correlation of Glc H-1″ (δ_H 5.37) / Fru C-2‴ (δ_C 106.8), and later by TLC analysis of alkaline hydrolysis product of **1** in comparison with authentic sucrose [16, 18]. Assignments of sucrose backbone were then elucidated by two set of individual COSY cross peaks Glc H-1″ (δ_H 5.37)/ Glc H-2″ (δ_H 4.43)/ Glc H-3″ (δ_H 3.74)/ Glc H-4″ (δ_H 3.13)/ Glc H-5″ (δ_H 4.05)/ Glc H-6″ (δ_H 3.83 and 4.85) and Fru H-3‴ (δ_H 4.23)/ Fru H-4‴ (δ_H 4.03)/ Fru H-5‴ (δ_H 4.07)/ Fru H-6‴ (δ_H 4.22 and 4.33). Connection

between sucrose moiety and truxinic acid derivative was deduced by ester linkages at Glc C-6″ and Fru C-6‴ which supported by HMBC correlations of Glc H-6″ (δ_H 3.83 and 4.85)/ C-9 (δ_C 174.5) and Fru H-6‴ (δ_H 4.22 and 4.33)/ C-9′ (δ_C 174.2). Finally, stereogenic centers at truxinic acid backbone were elucidated by NOESY (Figure 3). The NOESY spectrum of **1** exhibited the correlations between H-2 (δ_H 6.43)/ H-6 (δ_H 6.66) and H-8 (δ_H 3.88), H-2′ (δ_H 6.34)/ H-6′ (δ_H 6.48) and H-8′ (δ_H 3.97), indicating *trans*-orientations between C-7 and C-8, C-7′ and C-8′, respectively. The NOESY correlation between H-2 (δ_H 6.43) and H-8′ (δ_H 3.97) also indicated *trans*-orientation between C-7 and C-8′. Thus, relative configurations at truxinic acid backbone were proposed to be 7*α*,8*β*,7′*α*,8′*β* orientations. Consequently, structure of compound **1** was established as presented in Figure 1 and was named as trigohonbanoside A.

The HR-ESI-MS analysis of compound **2** exhibited a pseudo-molecular ion peak at *m/z* 729.1798 $[M+Cl]^-$, suggesting that compound **2** had the same molecular formula, $C_{32}H_{38}O_{17}$, with compound **1**. The ^{1}H- NMR of **2** (Table 1) observed similar pattern with those of **1** such as a pair of ABX coupled spin system [δ_H 6.42 (d, *J* = 1.5 Hz, H-2), 6.68 (d, *J* = 8.5 Hz, H-5), 6.69 (dd, *J* = 1.5 and 8.5 Hz, H-6), 6.34 (d, *J* = 2.0 Hz, H-2′), 6.60 (d, *J* = 9.0 Hz, H-5′), 6.50 (dd, *J* = 2.0 and 9.0 Hz, H-6′)], two methoxy groups [δ_H 3.62 (s, 3-OC$\underline{H}_3$) and 3.61 (s, 3′-OC$\underline{H}_3$)], an anomeric proton [δ_H 5.35 (d, *J* = 3.5 Hz, Glc H-1″)], and four aliphatic protons [δ_H 4.29 (dd, *J* = 4.5 and 10.0 Hz, H-7), 3.89 (dd, *J* = 4.5 and 10.5 Hz, H-8), 4.24 (dd, *J* = 9.0, 10.0 Hz, H-7′), 4.10 (dd, *J* = 9.0 and 10.5 Hz, H-8′)]. The COSY cross peaks of H-7/H-8/H-8′/H-7′/H-7 revealed the presence of cyclobutan ring. The HMBC correlations between H-2 (δ_H 6.42)/ H-6 (δ_H 6.69) and C-4 (δ_C 146.1), H-5 (δ_H 6.68)/ 3-OC$\underline{H}_3$ (δ_H 3.62) and C-3 (δ_C 148.6), H-2′ (δ_H 6.34)/ H-6′ (δ_H 6.50) and C-4′ (δ_C 146.1), H-5′ (δ_H 6.60)/ 3′-OC$\underline{H}_3$ (δ_H 3.61) and C-3′ (δ_C 148.5), H-7 (δ_H 4.29) and C-1 (δ_C 131.7)/ C-2 (δ_C 114.1)/ C-6 (δ_C 121.4), H-7′ (δ_H 4.24) and C-1′ (δ_C 131.6)/ C-2′ (δ_C 113.2)/ C-6′ (δ_C 121.6), H-8

(δ_H 3.89)/ H-8′ (δ_H 4.10) and carbonyl carbons C-9/C-9′ (δ_C 174.2) indicated the presence of 4,4′-dihydroxy-3,3′-dimethoxy-truxinic acid. An anomeric methine (δ_C 92.8 /δ_H 5.35), non-protonated anomeric carbon (δ_C 105.6), and HMBC correlation of Glc H-1″ (δ_H 5.35)/ Fru C-2‴ (δ_C 105.6) also suggested that sugar moiety of **2** also to be sucrose. The HMBC correlations between Glc H-6″ (δ_H 4.58 and 4.02)/C-9 (δ_C 174.2) and Fru H-6‴ (δ_H 4.56 and 4.36)/ C-9′ (δ_C 174.2) confirmed sucrose moiety binding with 4,4′-dihydroxy-3,3′-dimethoxy-truxinic acid by ester linkages at Glc C-6″ and Fru C-6‴. Interestingly, the NOESY spectrum of **2** observed correlations between H-2 (δ_H 6.42)/ H-6 (δ_H 6.69) and H-8 (δ_H 3.89), H-2′ (δ_H 6.34)/ H-6′ (δ_H 6.50) and H-8′ (δ_H 4.10), H-2 (δ_H 6.42) and H-8′ (δ_H 4.10) which are identical with that of **1**. Above mentioned NMR evidence indicated that truxinic acid moiety of **1** and **2** are a pair of enantiomers. This deduction was further confirmed by opposite trend of Cotton effects of **2** (+21.5 at wavelength of 245 nm) compared to **1** (−19.1 at wavelength of 244 nm, Figure 4). Thus, configurations at truxinic acid backbone of compound **2** were determined to be 7β,8α,7′β,8′α. The structure of **2** was then established and named as trigohonbanoside B.

The molecular formula of **3** was deduced to be $C_{31}H_{36}O_{16}$ based on the pseudo-molecular ion peak at *m/z* 699.1697 $[M+Cl]^-$ in the HR-ESI-MS (calcd. for $C_{31}H_{36}O_{16}Cl$, 699.1692). The ^{1}H-NHR spectrum of **3** exhibited an AA′BB′ spin system [δ_H 6.93 and 6.61 (each 2H, d, J = 8.5 Hz)] and an ABX spin system [δ_H 6.31 (1H, d, J = 2.0 Hz), 6.57 (1H, d, J = 8.0 Hz), 6.45 (1H, dd, J = 8.0 and 2.0 Hz)] instead of a pair of ABX spin systems as in the compounds **1** and **2**. Careful comparison ^{1}H- and ^{13}C-NMR spectral data of **3** with those of compounds **1**-**2** recognized that NMR data of **3** close similarity with compound **2** except signal of A-benzen ring (Table 1 and Figure 1). Appearance of deshielded carbon signal [δ_C 156.9 (C-4)] together with HMBC correlations

between H-2/H-6 (δ_H 6.93) and C-4 indicated that A-benzene ring of **3** to be 4-hydroxyphenyl group which was difference with 3-methoxy-4-hydroxyphenyl group in compound **2**. NOESY correlations between H-2/H-6 (δ_H 6.93) and H-8 (δ_H 3.85), H-2′ (δ_H 6.31)/ H-6′ (δ_H 6.45) and H-8′ (δ_H 4.11) indicated *trans*-orientations between C-7 and C-8, C-7′ and C-8′, respectively. The NOESY correlation between H-2 (δ_H 6.93) and H-8′ (δ_H 4.11) also indicated *trans*-orientation between C-7 and C-8′. Furthermore, CD spectrum of compound **3** (+13.9 at wavelength of 243 nm) showed similar Cotton effect with compound **2** (Figure 4), suggesting the identical stereogenic configuration at truxinic acid moiety between compounds **3** and **2**. Consequently, compound **3** was determined and named as trigohonbanoside C.

The molecular formula of compound **4** was identified as $C_{31}H_{36}O_{16}$ based on the HR-ESI-MS at *m/z* 699.1710 $[M+Cl]^-$ (calcd. for $C_{31}H_{36}O_{16}Cl$, 699.1692). The NMR spectral data of **4** also indicated it to be a sucrose ester of substituted truxinic acid, showing signals of sucrose moiety [twelve oxygenated carbons at δ_C 63.7 ~ 105.7, one anomeric proton δ_H 5.35 (1H, d, J = 3.5 Hz)], signals of cyclobutan ring [four aliphatic carbons (δ_C 45.9, 45.1, 44.4, 44.6) and their binding protons (δ_H 4.29, 3.86, 4.23, 4.10)], twelve aromatic carbons (δ_C 114.0 ~ 157.0), and two carbonyl carbons (δ_C 174.3 and 174.4). Like compound **3**, signals of a de-shielded carbon (δ_C 157.0) and AA′BB′ coupled protons [δ_H 6.78 and 6.56 (each, 2H, d, J = 8.5 Hz)] indicated the presence of 4-hydroxyphenyl group (B-benzen ring). Due to overlapped signals between H-5 and H-6 (δ_H 6.64), the pattern of A-benzene ring is hard to recognize in the ^{1}H-NMR spectrum. However, the HMBC correlations between H-2 (δ_H 6.40)/ H-6 (δ_H 6.64) and C-4 (δ_C 146.0), H-5 (δ_H 6.64)/ 3-OC$\underline{H}_3$ (δ_H 3.62) and C-3 (δ_C 148.5), NOESY correlation between H-2 (δ_H 6.40) and 3-OC$\underline{H}_3$ (δ_H 3.62) confirmed the A benzene ring to be 3-methoxy-4-hydroxyphenyl group. NOESY correlations between H-2 (δ_H 6.40)/H-6 (δ_H 6.64) and H-8 (δ_H 3.86), H-2′/H-6′ (δ_H 6.78) and H-8′ (δ_H 4.10)

indicated *trans*-orientations between C-7 and C-8, C-7′ and C-8′, respectively. The NOESY correlation between H-2 (δ_H 6.40) and H-8′ (δ_H 4.10) also indicated *trans*-orientation between C-7 and C-8′. Furthermore, CD spectrum of compound **4** (+18.1 at wavelength of 238 nm) showed similar Cotton effect with compounds **2-3** (Figure 4), suggesting the identical stereogenic configurations at truxinic acid moiety between compounds **2-4**. Consequently, compound **4** was determined and named as trigohonbanoside D.

Truxinic acid derivatives are reported naturally arising via head-to-head [2+2]-cyclodimerization of cinnamic acid derivatives. Cyclodimeric products formed by making C-C bonds between C-7 and C-7′, C-8 and C-8′ [19]. Their chemical structures therefore can be considered as dimeric derivatives of phenylpropanoid and also called as truxinate lignans by some authors [20]. Compounds **1** and **2** could be generated from cyclodimeric of two caffeic acid moieties meanwhile compounds **3** and **4** were expected to form by [2+2]-cycloaddition between a caffeic acid moiety and a coumaric acid moiety. Relative configurations at cyclobutane-ring of compounds **1-4** were similar. However, CD spectral data indicated that absolute configurations at cyclobutane-ring of compound **1** were mirror-image with those of compounds **2-4** as described in the Figure 1.

ANO1 is known as transmembrane protein 16A which is identified to be a Ca^{2+}-activated chloride channel expressing in various cell types. It recently considered as an important marker involved in the regulation of cell proliferation, smooth muscle contraction, epithelial fluid secretion. Therefore, ANO1 inhibitors would be beneficial to treatments of related diseases such as cancer, hypertension, asthma, and pain [15, 21, 22]. Recent study indicates natural phenolics could be good ANO1 inhibitors [15]. Compounds **1-4** were then screened for their effect on ANO1 activity. At concentration of 30 μM, compounds **1-4** moderately inhibited ANO1 activity with inhibitory percentages of 27.7 ± 1.10 %, 35.6 ± 0.92 %, 43.7 ± 1.61 %, and 40.8 ± 1.25%, respectively. The

most potent inhibitor, 2-(4-chloro-2-methylphenoxy)-*N*-[(2-methoxyphenyl)methylideneamino]-acetamide (Ani9) was used at 3 μM as a positive control, showing inhibitory rate of 97.5 ± 0.34 %. Significant ANO1 inhibitory activity of compounds **1-4** were consisted with previous reports that natural phenolics could be good ANO1 inhibitors [15].

Declaration of Competing Interest

The authors declare no conflict of interest.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https:// doi.org/…

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Figure 1. Structures of compounds **1-4** isolated from *T. honbaensis*

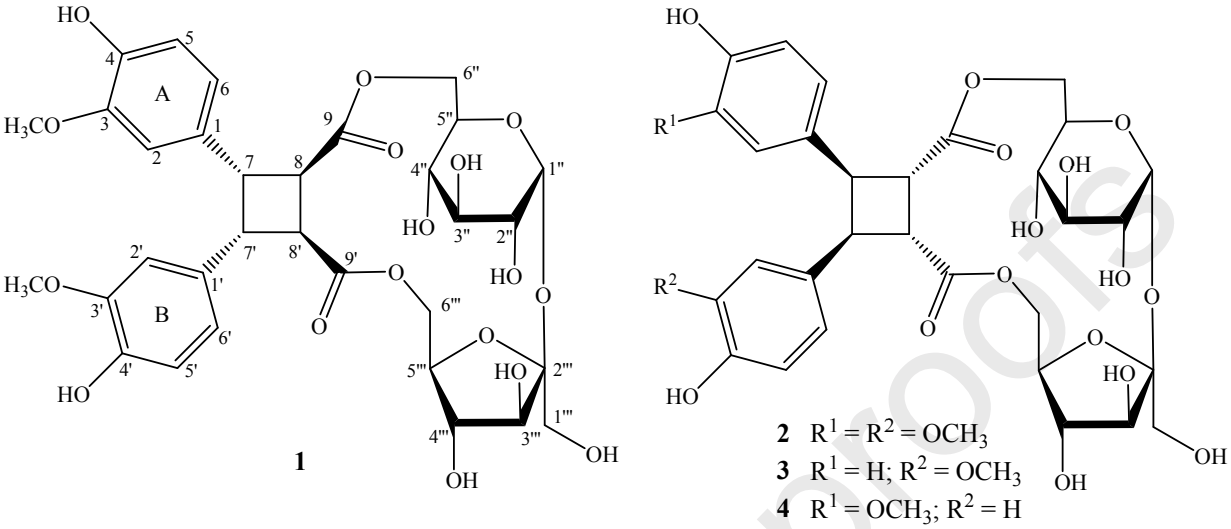

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Figure 2. Key HMBC (H → C) and COSY (H ▬ H) correlations of compounds **1-4**

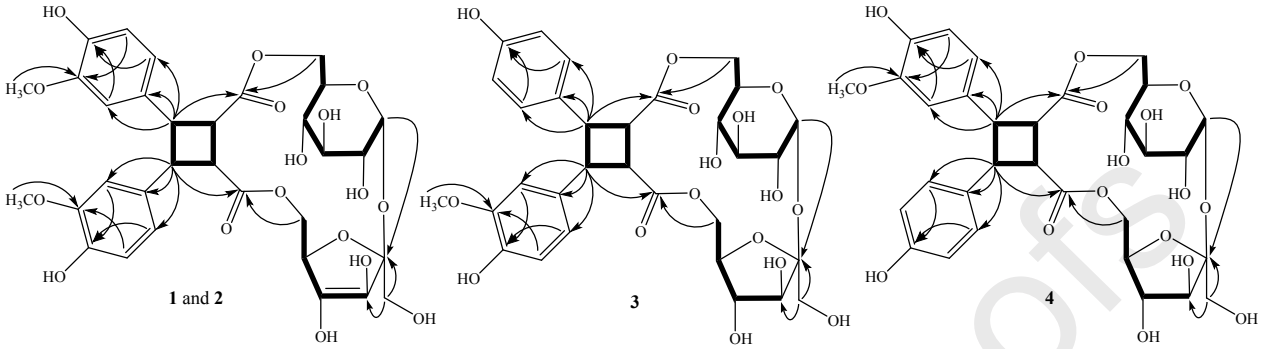

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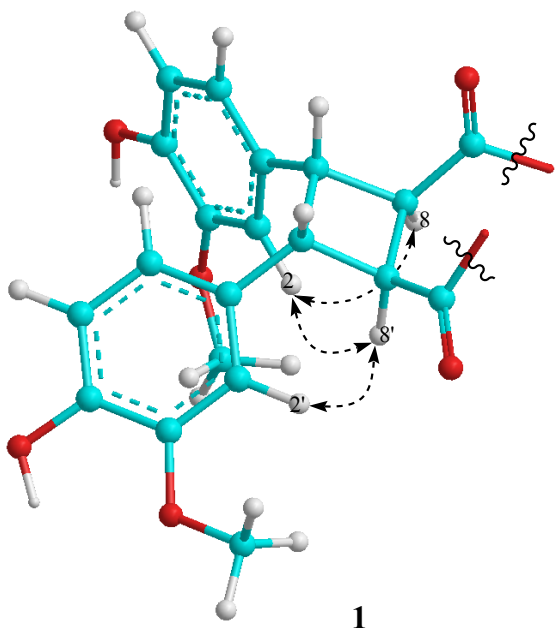
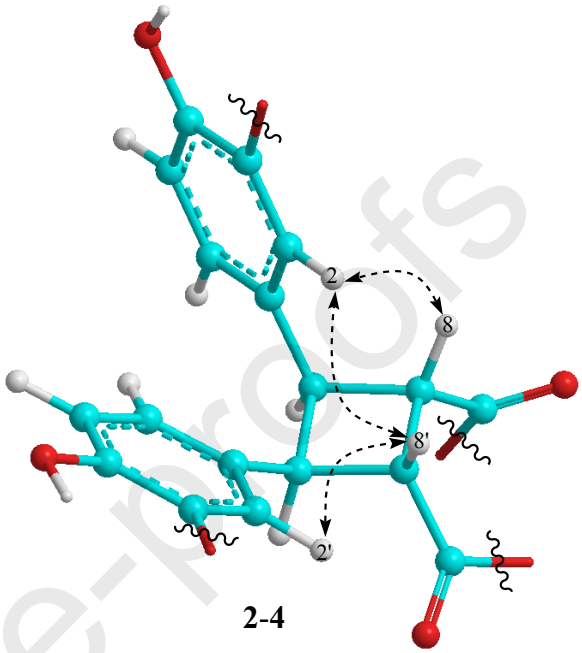
Figure 3. Important NOESY correlations in aglycone moieties of compounds **1-4**



1



2-4

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Figure 4. Circular dichroism spectra of compounds **1-4**

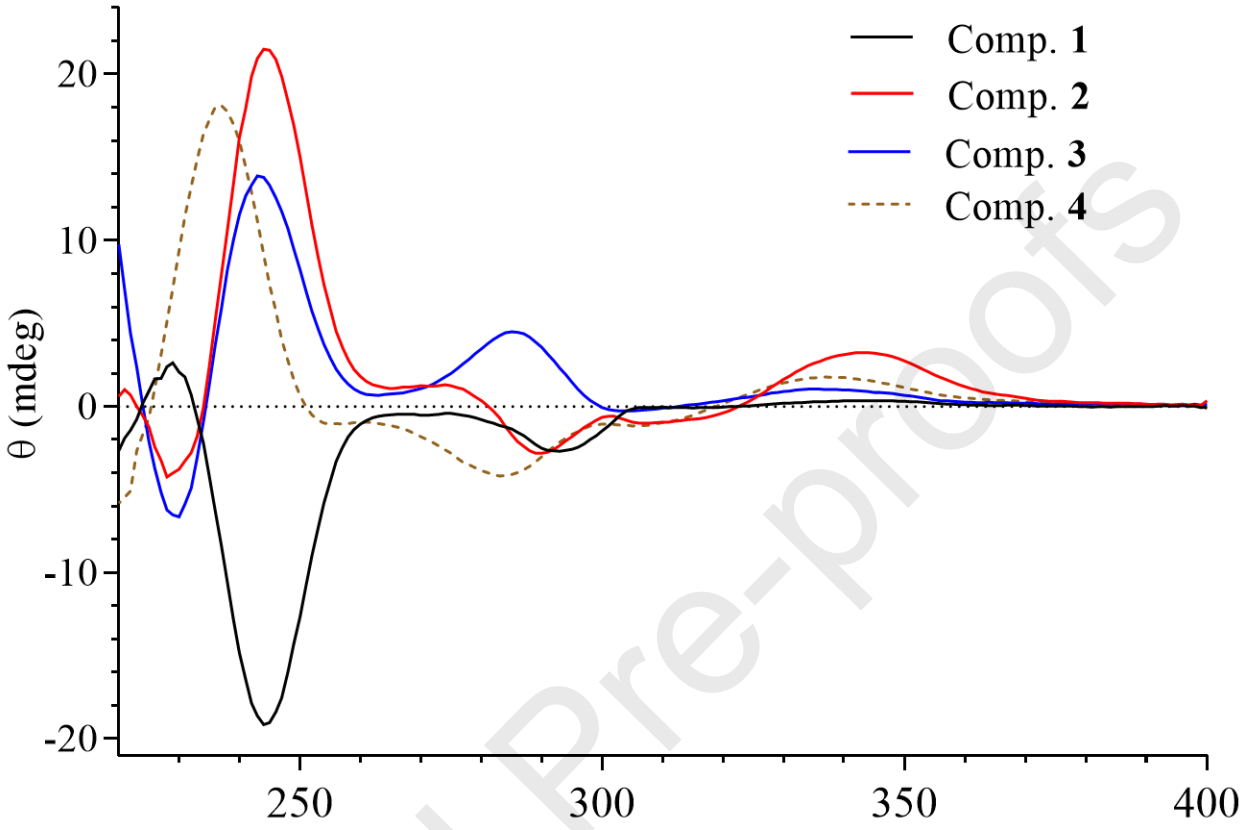

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Table 1. ^{1}H and ^{13}C-NMR spectral data of compounds **1-4**

No.	1		2		3		4	
	[a,b]δ_C	[a,c]δ_H (mult., *J* in Hz)	[a,b]δ_C	[a,c]δ_H (mult., *J* in Hz)	[a,b]δ_C	[a,c]δ_H (mult., *J* in Hz)	[a,b]δ_C	[a,c]δ_H (mult., *J* in Hz)
1	131.8	-	131.7	-	131.0	-	131.7	-
2	113.7	6.43 (d, 2.0)	114.1	6.42 (d, 1.5)	130.4	6.93 (d, 8.5)	114.0	6.40 (br s)
3	148.7	-	148.6	-	115.9	6.61 (d, 8.5)	148.5	-
4	146.1	-	146.1	-	156.9	-	146.0	-
5	115.7	6.66 (d, 8.5)	115.7	6.68 (d, 8.5)	115.9	6.61 (d, 8.5)	115.7	6.64*
6	121.4	6.61 (dd, 2.0, 8.5)	121.4	6.69 (dd, 1.5, 8.5)	130.4	6.93 (d, 8.5)	121.4	6.64*
7	46.2	4.20 (dd, 5.0, 10.0)	45.9	4.29 (dd, 4.5, 10.0)	45.6	4.28 (dd, 4.5, 10.0)	45.9	4.29 (5.0, 9.5)
8	44.8	3.88 (dd, 5.0, 10.5)	44.8	3.89 (dd, 4.5, 10.5)	45.1	3.85 (dd, 4.5, 10.0)	45.1	3.86 (5.0, 10.0)
9	174.5	-	174.2	-	174.2	-	174.4	-
1′	131.7	-	131.6	-	131.7	-	131.0	-
2′	113.1	6.34 (d, 2.0)	113.2	6.34 (d, 2.0)	113.0	6.31 (d, 2.0)	130.1	6.78 (d, 8.5)
3′	148.5	-	148.5	-	148.4	-	115.8	6.56 (d, 8.5)
4′	146.1	-	146.1	-	146.0	-	157.0	-
5′	115.6	6.60 (d, 8.5)	115.6	6.60 (d, 9.0)	115.5	6.57 (d, 8.0)	115.8	6.56 (d, 8.5)
6′	121.6	6.48 (dd, 2.0, 8.5)	121.6	6.50 (dd, 2.0, 9.0)	121.6	6.45 (dd, 2.0, 8.0)	130.1	6.78 (d, 8.5)
7′	45.2	4.25 (dd, 8.5, 10.0)	44.9	4.24 (dd, 9.0, 10.0)	44.7	4.23 (dd, 8.5, 10.0)	44.4	4.23 (dd, 8.5, 9.5)
8′	44.7	3.97 (dd, 8.5, 10.5)	44.8	4.10 (dd, 9.0, 10.5)	45.0	4.11 (dd, 8.5, 10.0)	44.6	4.10 (dd, 8.5, 10.0)
9′	174.2	-	174.2	-	174.2	-	174.3	-
3-OCH_3	56.4	3.63 (s)	56.4	3.62 (s)	-	-	56.4	3.62 (s)
3′-OCH_3	56.3	3.61 (s)	56.3	3.61 (s)	56.3	3.61 (s)	-	-
1″	94.7	5.37 (d, 3.5)	92.8	5.35 (d, 3.5)	92.8	5.35 (d, 3.5)	92.9	5.35 (d, 3.5)
2″	73.4	4.43 (dd, 3.5, 9.5)	73.0	4.43 (dd, 3.5, 9.5)	73.0	3.48 (dd, 3.5, 9.5)	73.0	3.47 (dd, 3.5, 9.5)
3″	74.1	3.74 (dd, 9.5, 9.5)	74.6	3.76 (dd, 9.5, 9.5)	74.6	3.75 (dd, 9.5, 9.5)	74.5	3.66 (t, 9.5)
4″	73.1	3.13 (dd, 9.5, 9.5)	72.6	3.12 (dd, 9.5, 9.5)	72.6	3.12 (dd, 9.0, 9.5)	72.6	3.12 (dd, 9.0, 9.5)
5″	73.0	4.05 (m)	72.5	4.15 (m)	72.4	4.16 (m)	72.5	4.16 (m)
6″	67.6	3.83 (dd, 9.0, 11.5) 4.85 (br d, 11.5)	68.6	4.02 (dd, 9.0, 11.5) 4.58 (dd, 2.5, 11.5)	68.6	4.00 (dd, 8.5, 12.0) 4.57 (dd, 1.5, 12.0)	68.5	4.03 (dd, 9.0, 11.5) 4.57 (dd, 1.5, 11.5)
1‴	61.9	3.87 (s)	63.8	3.67 (s)	63.8	3.66 (s)	63.7	3.66 (s)
2‴	106.8	-	105.6	-	105.6	-	105.7	-
3‴	79.4	4.23 (d, 7.5)	79.2	4.14 (d, 8.0)	79.2	4.14 (d, 7.5)	79.2	4.15 (d, 7.5)
4‴	77.7	4.03 (dd, 7.5, 7.5)	77.9	4.31 (dd, 8.0, 8.0)	77.9	4.32 (dd, 7.5, 7.5)	77.9	4.30 (dd, 7.5, 7.5)
5‴	80.7	4.07 (m)	79.2	4.16 (m)	79.2	4.16 (m)	79.2	4.17 (m)
6‴	68.4	4.22 (br d, 10.5) 4.33 (dd, 10.5, 10.5)	67.5	4.36 (dd, 8.0, 10.5) 4.56 (dd, 8.5, 10.5)	67.5	4.36 (dd, 8.0, 10.5) 4.56 (dd, 8.5, 10.5)	67.5	4.36 (dd, 8.0, 10.5) 4.56 (dd, 8.5, 10.5)

Measured in [a]CD_3OD, [b]125 MHz, [c]500 MHz, *)Overlapped signals.

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