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Xanthones from the twigs of *Garcinia oblongifolia* and their antidiabetic activity

Binh T.D. Trinh [a,b], Tam T.T. Quach [b], Dung N. Bui [b], Dan Staerk [a,*], Lien-Hoa D. Nguyen [b,*], Anna K. Jäger [a]

[a] Department of Drug Design and Pharmacology, Faculty of Health and Medical Sciences, University of Copenhagen, Universitetsparken 2, DK 2100 Copenhagen, Denmark
[b] Natural Product and Medicinal Chemistry Lab, Faculty of Chemistry, Ho Chi Minh City University of Science, 227 Nguyen Van Cu, Ho Chi Minh City, Vietnam

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Cowanin (PubChem CID: 11754819)
Cowanol (PubChem CID: 101671063)
Rubraxanthone (PubChem CID: 9953366)
Cowagarcinone E (PubChem CID: 11421303)
Norcowanin (PubChem CID: 11518330)
Acarbose (PubChem CID: 444254)
RK682 (PubChem CID: 54678922)

ABSTRACT

Three new xanthones, oblongixanthone F–H (**1**–**3**), along with eight known xanthones (**4**–**11**), were isolated from an EtOAc extract of the twigs of *Garcinia oblongifolia*. Their structures were elucidated by spectroscopic analysis including 1D- and 2D-NMR spectroscopy and mass spectrometry. The antidiabetic effects of all isolated compounds were evaluated by in vitro α-glucosidase and PTP1B inhibition assays. Compound **11** was the most active compound, and inhibited α-glucosidase and PTP1B with IC_{50} values of 1.7 ± 0.5 and 14.1 ± 3.5 μM, respectively.

1. Introduction

Diabetes mellitus is a metabolic disorder that has become increasingly common worldwide. In 2015, 415 million people were affected, but this number is predicted to increase to 642 million people by 2040. The burden of diabetes is enormous, and caused 5 million deaths and amounted between 673 and 1197 billion US dollars in health expenditures in 2015 [1]. More than 90% of diabetes cases are classified as type 2 diabetes, which is characterized by insulin resistance [2]. This results in postprandial hyperglycemia causing severe long-term complications, such as diabetic neuropathy, diabetic retinopathy, diabetic nephropathy and cardiovascular diseases [3–5]. Various pharmacological approaches have been used to alleviate diabetes such as stimulation of insulin secretion, improvement of insulin sensitivity, and reduction of absorption of glucose from the intestine [6–8]. The goal for treatment of type 2 diabetes mellitus is generally maintenance of glycemic control in the postprandial state and the fasting state [9].

α-Glucosidase is an enzyme located in the brush border of the small intestine. This enzyme catalyzes the hydrolysis of the α-glycosidic bond of oligosaccharides to liberate the monosaccharide units which are absorbed into the blood stream [10]. Thus, inhibition of α-glucosidase is an effective way to delay the uptake of dietary carbohydrates and suppress postprandial hyperglycemia. The insulin-signaling pathway is one of the key pathways responsible for blood glucose regulation. Insulin action is initiated by insulin binding to its receptor, eliciting receptor autophosphorylation and activation of the receptor tyrosine kinase, resulting in tyrosine phosphorylation of insulin receptor substrates (IRS). This leads to a series of downstream signaling events which is an important step for translocation of glucose transporter 4 (GLUT4) from an intracellular site to the cell surface to allow glucose uptake into the muscle. In this pathway, the enzyme protein-tyrosine phosphatase 1B (PTP1B) acts as a negative regulator which can dephosphorylate, and thereby, deactivate the insulin receptor. Therefore, PTP1B inhibitors have been proposed as potential drugs for the treatment of type 2

* Corresponding authors.
E-mail addresses: ds@sund.ku.dk (D. Staerk), lienhoa-nguyen@vnn.vn (L.-H.D. Nguyen).

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diabetes [11,12]. In our ongoing search for natural antidiabetic agents, the EtOAc extract of the twigs of *Garcinia oblongifolia* was found to possess α-glucosidase and PTP1B inhibitors. *Garcinia*, one of the biggest genera of the family Guttiferae with approximately 300 species [13], has been found to be a prolific source of xanthones, flavonoids, benzophenones and triterpenoids, of which xanthones are the most abundant [14–16]. Phenolic constituents from *Garcinia* species have been reported to possess various biological activities, including anti-inflammatory, antibacterial, antioxidant, and anti-HIV properties [17–20]. We now report the isolation, structural elucidation, and antidiabetic activity of compounds from the EtOAc extract of *G. oblongifolia*.

2. Experimental

2.1. General experimental procedures

UV spectra were recorded on an Agilent 8453 spectrophotometer. HRESIMS spectra were measured on a Bruker micrOTOF-QII MS (80 eV). Column chromatography (CC) was run on silica gel (Merck, 40–63 μm) or RP_{18} (Merck, 40–63 μm). For gel permeation chromatography (GPC), Sephadex LH-20 (GE Healthcare) with $CHCl_3$–MeOH 1:1 as eluent was used. TLC was carried out on precoated glass TLC plates normal phase (Merck, 250 μm) and RP_{18} (Merck, 200 μm). Spots on TLC were visualized using UV light, staining with I_2 or spraying with $FeCl_3$/EtOH or detection of phenolic compounds.

2.2. Plant material

The twigs of *G. oblongifolia* were collected in Song Kon Plantation, Binh Dinh Province and identified by Dr. Dang Van Son, Institute of Tropical Biology, Ho Chi Minh City. A voucher specimen (Bua LTD-SongKon) is deposited in the Natural Product and Medicinal Chemistry Lab, Ho Chi Minh City University of Science.

2.3. Extraction and isolation

The air-dried and ground twigs of *G. oblongifolia* (3.4 kg) were exhaustively extracted with 7.5 L of EtOAc using a Soxhlet extractor. Evaporation of the solvent produced an EtOAc extract (125 g), which was fractionated over a silica gel column chromatography (CC) (0–100% acetone in hexane) to give 20 fractions (volume of each collected fraction was 1 L), which were then combined into nine fractions (Fr.1–9) based on TLC.

Fraction 5 (1.42 g) was chromatographed on GPC (2.7 L of eluent; volume of each collected fraction was 100 mL) to get 10 fractions (Fr.5.1–10). Compound **4** (6.4 mg) was isolated from fraction 5.7 (35 mg) using CC (0–40% acetone in hexane). CC of fraction 5.9 (67 mg) on RP_{18} (60–100% MeOH in H_2O), followed by CC (0–30% EtOAc in hexane) yielded compound **5** (4.5 mg).

CC of fraction 6 (17.3 g) over silica gel (0–50% EtOAc in hexane, 5 L, volume of each collected fraction was 200 mL) yielded 10 fractions (Fr.6.1–10). Further CC of fraction 6.5 (3.5 g) with the same eluent (3 L, volume of each collected fraction was 100 mL) afforded 10 fractions (Fr.6.5.1–10). Fraction 6.5.2 (260 mg) was separated using CC (60–100% $CHCl_3$ in hexane, 1.5 L, volume of each collected fraction was 50 mL), which afforded 10 fractions (Fr.6.5.2.1–10). Purification of fraction 6.5.2.3 (24.6 mg) using GPC led to the isolation of compound **1** (18.5 mg) while CC of fraction 6.5.2.7 (33.3 mg) (0–30% EtOAc in hexane) obtained compound **6** (8 mg). Fraction 6.5.3 (1.2 g) was subjected to CC (0–30% EtOAc in hexane, 2.3 L, volume of each collected fraction was 100 mL) to yield 13 fractions (Fr.6.5.3.1–13). Compound **2** (9 mg) was isolated from fraction 6.5.3.9 (69 mg) using RP_{18} (60–100% acetone in H_2O). Repeated CC of fraction 6.8 (2.8 g) on silica gel (0–50% EtOAc in $CHCl_3$ followed by 0–30% EtOAc in $CHCl_3$), followed by GPC furnished compound **3** (10 mg). Fraction 6.9 (385 mg) was separated using CC (0–30% EtOAc in $CHCl_3$) and then purified using GPC to give compound **7** (77 mg).

Fraction 8 (30.5 g) was subjected to CC (0–50% EtOAc in hexane, 7 L, volume of each collected fraction was 500 mL) to afford 9 fractions (Fr.8.1–9). CC of fraction 8.5 (5.0 g) (0–50% EtOAc in hexane) led to the isolation of compound **8** (5 mg). Fraction 8.7 (3.5 g) was CC (0–50% EtOAc in hexane and 0–30% EtOAc in hexane, respectively) and finally purified using RP_{18} (60–100% acetone in H_2O) to obtain compound **9** (9 mg).

Fraction 9 (17.8 g) was partitioned by CC (0–50% EtOAc in hexane, 4.4 L, volume of each collected fraction was 200 mL) to give 7 fractions (Fr.9.1–7). Further CC fraction of 9.4 (2.8 g) (0–50% EtOAc in hexane, 2.9 L, volume of each collected fraction was 100 mL) afforded 10 fractions (Fr.9.4.1–10). Fraction 9.4.3 was separated using CC (0–30% EtOAc in $CHCl_3$) and then GPC to furnish compound **10** (6.0 mg). Meanwhile, fraction 9.4.7 was separated using CC (0–10% isopropanol in hexane) and then GPC to obtain compound **11** (7 mg).

2.3.1. Olongixanthone F (compound *1*)

Yellow crystals; UV (EtOH) λ_{max} 247, 263, 325, and 369 nm; HRESIMS *m/z* 555.2719 $[M + Na]^+$ (calcd for $C_{33}H_{40}O_6Na^+$, 555.2717); ^1H and ^{13}C NMR data: Table 1.

2.3.2. Olongixanthone G (compound *2*)

Yellow crystals; UV (EtOH) λ_{max} 238, 256, 284, and 332 nm; HRESIMS *m/z* 433.1621 $[M + Na]^+$ (calcd for $C_{24}H_{26}O_6Na^+$, 433.1622); ^1H and ^{13}C NMR data: Table 1.

2.3.3. Olongixanthone H (compound *3*)

Yellow crystals; UV (EtOH) λ_{max} 243, 260, 317, and 362 nm; HRESIMS *m/z* 477.2252 $[M + H]^+$ (calcd for $C_{29}H_{33}O_6^+$, 477.2272); ^1H and ^{13}C NMR data: Table 1.

2.4. Spectrophotometric microplate-based in vitro α-glucosidase inhibition assay

Inhibition of yeast α-glucosidase was assessed by spectrophotometric measurement of the cleavage rate of *p*-nitrophenyl β-D-glucopyranoside (PNPG) to the strongly chromogenic *p*-nitrophenolate ion as described by Schmidt et al. [21]. In brief, 90 μL of 0.1 M phosphate buffer (pH 7.5, 0.02% NaN_3), 10 μL test sample dissolved in DMSO, and 80 μL of enzyme solution (final well concentration 0.05 U/mL) were added to each well of a 96-well microplate. The mixture was incubated at 28 °C for 10 min before adding PNPG to a final volume of 200 μL (final well concentration 1.0 mM). The hydrolysis rate of PNPG to *p*-nitrophenolate was monitored at 405 nm every 30 s for 35 min. Incubation and absorbance measurements were performed with a Multiscan FC microplate photometer with built-in incubator (Thermo Scientific, Waltham, MA), controlled by SkanIt ver. 2.5.1 software for data acquisition. The α-glucosidase inhibitory activity was expressed as percentage inhibition and was calculated using the following formula:

$$\%\text{inhibition} = \left(\text{Slope}_{\text{blank}} - \text{Slope}_{\text{sample}}\right)/\text{Slope}_{\text{blank}} \times 100$$

Acarbose was used as positive control and all measurements were performed in triplicate. Dose-response curves and IC_{50} values were obtained using GraFit Version 5 (Erithacus Software Ltd., Horley, UK).

2.5. Spectrophotometric microplate-based in vitro PTP1B inhibition assay

Inhibition of PTP1B was assessed by spectrophotometric measurement of the cleavage rate of *p*-nitrophenyl phosphate (pNPP) to the strongly chromogenic *p*-nitrophenolate ion as described by Tahtah et al. [22]. The PTP1B inhibition assay was performed at 25 °C in 96-well microplates using a two-component buffer, containing 50 mM Tris,