

## Research Article

Tin Myo Thant, Nanik Siti Aminah\*, Alfinda Novi Kristanti, Rico Ramadhan, Hnin Thanda Aung, Yoshiaki Takaya

# New derivatives of a natural nordentatin

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**Abstract:** New derivatives were obtained from natural nordentatin (**1**) previously isolated from the methanol fraction of *Clausena excavata* by an acylation method. Herein, we report ten new pyranocoumarin derivatives **1a–1j**. Their structures were elucidated based on UV-vis, FT-IR, NMR, and DART-MS data. The  $\alpha$ -glucosidase inhibition and anticancer activities of nordentatin (**1**) and its derivatives were also evaluated. The  $\alpha$ -glucosidase inhibition assay exhibited that the derivatives **1b**, **1d**, **1e**, **1f**, **1h**, **1i**, and **1j** possess higher inhibitory activity for  $\alpha$ -glucosidase with  $IC_{50}$  values of 1.54, 9.05, 4.87, 20.25, 12.34, 5.67, and 2.43 mM, whereas acarbose was used as the positive control,  $IC_{50} = 7.57$  mM. All derivatives exhibited a weak cytotoxicity against a cervical cancer (HeLa) cell line with the  $IC_{50}$  between 0.25 and 1.25 mM. They also showed moderate to low growth inhibition of a breast cancer (T47D) cell line with  $IC_{50}$  values between 0.043 and 1.5 mM, but their activity was lower than that of the parent compound, nordentatin (**1**) ( $IC_{50} = 0.041$  mM).

**Keywords:** *Clausena excavata*, pyranocoumarin derivatives, nordentatin, yeast  $\alpha$ -glucosidase inhibition, cytotoxicity, cervical cancer, breast cancer

## 1 Introduction

*Clausena excavata* Burm f. belongs to the Rutaceae family and grows mainly in south and southeast Asia [1]. The plant is a wild shrub; its leaves, twigs, and root barks are used as traditional medicine for the treatment of cold, malaria, abdominal pain, snake-bite, viral infections (e.g., HIV), and dermatopathy. The plant is reported to contain bioactive constituents with antibacterial, antifungal, antimycobacterial, antinociceptive, *in vivo* immunomodulating, and insecticidal properties. Phytochemical studies have revealed that *C. excavata* is a rich source of coumarins [2,3], carbazole alkaloids [4], and limonoid [5]. Coumarins are plant-derived compounds with a benzopyrone moiety. They possess a wide variety of biological activities. Coumarins and their derivatives are being extensively studied for their biological activities, low toxicity, and low drug resistance properties [6,7].

It has been reported that nordentatin exhibits hypoglycemic activity [8]. The conversion reaction of this compound into its ester derivative is desired, as the derivative is expected to possess more potent biological activities, especially as an antidiabetic medicine in the future. Diabetes mellitus is a chronic disease caused by the dysfunction of carbohydrate metabolism. It has been recognized as one of the most serious public health problems globally. The International Diabetes Federation has estimated that 425 million people worldwide have diabetes. The number will increase to 642 million by 2040 [9–11]. Recently, there has been immense interest in the study of enzymes as drug targets. Especially, the inhibition of  $\alpha$ -glucosidase can help in controlling postprandial glucose levels in diabetic patients.  $\alpha$ -Glucosidases ( $\alpha$ -D-glucoside glucohydrolase EC. 3.2.1.20) are membrane-bound enzymes, located in the epithelium of the small intestine, and the key enzymes of carbohydrate digestion. The inhibitors of  $\alpha$ -glucosidase can delay the digestion of starch and other dietary sugars, and thus they prevent the onset of hyperglycemia and maintain the normal blood sugar level [9–11]. Clinical trials have already shown that  $\alpha$ -glucosidase inhibitors can improve long-term glycemic

\* **Corresponding author: Nanik Siti Aminah**, Department of Chemistry, Faculty of Science and Technology, Airlangga University, Komplek Kampus C UNAIR, Jl. Mulyorejo, Surabaya, Indonesia, e-mail: nanik-s-a@fst.unair.ac.id, tel: +62-31-593-6501, fax: +62-31-593-6502

**Tin Myo Thant:** Department of Chemistry, Faculty of Science and Technology, Airlangga University, Komplek Kampus C UNAIR, Jl. Mulyorejo, Surabaya, Indonesia; Department of Chemistry, Mandalay Degree College, Mandalay, Myanmar

**Alfinda Novi Kristanti, Rico Ramadhan:** Department of Chemistry, Faculty of Science and Technology, Airlangga University, Komplek Kampus C UNAIR, Jl. Mulyorejo, Surabaya, Indonesia

**Hnin Thanda Aung:** Department of Chemistry, Mandalay University, Mandalay, Myanmar

**Yoshiaki Takaya:** Department of Pharmacy, Faculty of Pharmacy, Meijo University, 150 Yagotoyama, Tempaku, Nagoya, 468-8503, Japan

control and decrease hemoglobin A1c (HbA1c) levels in patients with type II diabetes. They can also delay the development of type II diabetes in patients with impaired glucose tolerance [12]. Inhibitors of  $\alpha$ -glucosidase such as voglibose, miglitol, and acarbose are currently used clinically, but their use is limited due to their adverse effects, such as diarrhea, abdominal cramping, flatulence, and vomiting. Therefore, much effort has been focused to develop effective  $\alpha$ -glucosidase inhibitors from natural sources [13–15].

Cancer, in its various forms, is one of the major causes of death of the human population. Among them, cervical and breast cancers are the most common causes of cancer-related deaths in women globally [15,17]. Several natural products of plant origin could potentially be used as cancer chemotherapeutic agents. Some of the currently used plant-derived anticancer agents include podophyllotoxin, taxol, vincristine, and camptothecin [17]. The conversion reaction of marchantin to its ester derivatives increases the anticancer activity [18]. In the present work, we semi-synthesized pyranocoumarin benzoate derivatives from nordentatin (**1**) by using different benzoyl chlorides. Subsequently, the inhibitory effects of the resulting derivatives and of the parent compound against  $\alpha$ -glucosidase enzymes and two cancer cell lines (HeLa and T47D) were evaluated and compared.

## 2 Materials and methods

### 2.1 Materials

#### 2.1.1 Materials for synthesis

NMR experiments were performed on Bruker 600 MHz ( $^1\text{H}$ ) and 151 MHz ( $^{13}\text{C}$ ) instruments in  $\text{CDCl}_3$  solvent. MS data were recorded on a DART-MS instrument. Melting points were determined on a Stuart (SMP30) instrument. Infrared spectra were recorded on an IR Tracer-100,  $\nu$  in  $\text{cm}^{-1}$ . The UV-vis spectra were obtained on a Shimadzu (UV-1800) UV-vis spectrometer. The target compounds (**1a–1j**) were semi-synthesized by an acylation method with slight modification [19,20]. The starting material, nordentatin (**1**), was collected from our previous research [2]. Reagents such as 4-bromobenzoyl chloride, 3-chlorobenzoyl chloride, 2-chloro-4-nitrobenzoyl chloride, 3-chloro-4-fluoro benzoyl chloride, 3-bromo-benzoyl chloride, 4-butylbenzoyl chloride, 2,4,6-trichlorobenzoyl chloride, 3-trifluoromethylbenzoyl chloride, 3,5-bis

(trifluoromethyl)benzoyl chloride, and 4-iodobenzoyl chloride (Wako, Japan) were used for the modification of compound **1** (nordentatin). Pyridine and 4-dimethylamino pyridine were used as catalysts. The reaction products were isolated by column chromatography on silica gel 60 (0.063–0.200 mm; Merck, Germany), by eluting with *n*-hexane and dichloromethane (20–100%). The reaction progress and the purity of the obtained compounds were monitored by p-TLC (preparative thin layer chromatography) on UV-254 plates (*n*-hex: $\text{CH}_2\text{Cl}_2$ , 9:1; detection under UV light and by spraying anisaldehyde, followed by heating). The solvents ( $\text{CH}_2\text{Cl}_2$ , *n*-hex, and EtOAc) were purified by standard methods and distilled just before use.

#### 2.1.2 Materials for antidiabetic and cytotoxic testing

The  $\alpha$ -glucosidase from *Saccharomyces cerevisiae* (EC 3.2.1.20), and 4-nitrophenyl  $\alpha$ -D-glucopyranoside (*p*-NPG) were obtained from Sigma-Aldrich. Spectrophotometric measurements for the yeast  $\alpha$ -glucosidase inhibition were recorded on an i-Mark microplate reader.

The materials used in the cytotoxicity test with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium-bromide (MTT) cell viability assay performed on cervical (HeLa) and breast (T47D) cancer cell lines are as follows: Dulbecco's Modified Eagle's Medium, fetal bovine serum (FBS) 10% (w/v), dimethyl sulfoxide, phosphate buffer solution, MTT, phenazine methosulfate, sodium dodecyl sulfate 10% (w/v), and HCl 0.1 N.

### 2.2 Methods

#### 2.2.1 General procedure for the modification of nordentatin (**1**) with benzoyl chloride derivatives by an acylation method

Esterification of nordentatin (**1**) was carried out by an acylation method [6,15] (Figure 1). First, around 100 mg of sample (**1**) was put in each flask. It was dissolved by adding 2 mL of pyridine and 10 mg of 4-(dimethylamino)-pyridine (DMAP) to the mixture. Subsequently, the mixture was stirred on a magnetic stirrer, and different kinds of benzoyl chlorides were added slowly. Then, the reaction was allowed to proceed at room temperature for 1 h. The mixture was checked with TLC at 30 min intervals until the reaction was completed. A



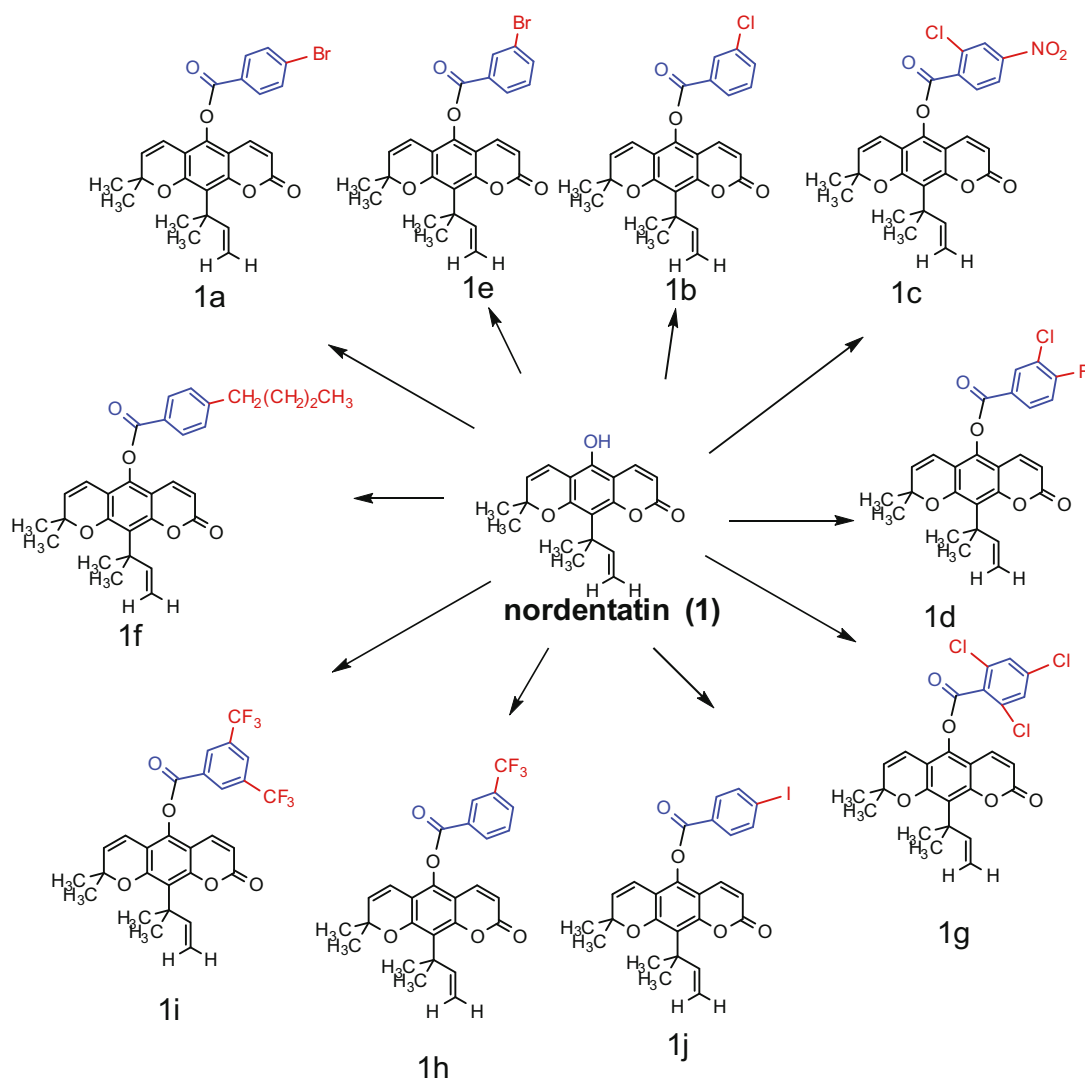


Figure 2: Structures of derivatives 1a–1j from nordentatin (1).

### 3.1.1 Spectral data of compound 1a, 2,2-dimethyl-10-(2-methylbut-3-en-2-yl)-8-oxo-2H,8H-pyrano[3,2-g]chromen-5-yl 4-bromobenzoate

Nordentatin (108.2 mg, 0.3467 mmol), 4-bromobenzoyl chloride (121.6 mg, 0.5500 mmol), yield 43.2%. UV (MeOH),  $\lambda_{\max}$  (log  $\epsilon$ ) 349 (0.79), 257 (2.87), 231 (1.0). M.p. 203–206°C. FT-IR 3,080, 2,972, 2,929, 2,873, 1,747, 1,730, 1,641, 1,618, 1,552  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR (chloroform-*d*, 600 MHz)  $\delta$  2  $\times$  8.11 (1H, d,  $J$  = 8.7 Hz), 2  $\times$  7.72 (1H, d,  $J$  = 8.7 Hz), 7.53 (1H, d,  $J$  = 9.7 Hz), 6.33 (1H, dd,  $J$  = 17.4, 10.6 Hz), 6.26 (1H, d,  $J$  = 10.0 Hz), 6.19 (1H, d,  $J$  = 9.7 Hz), 5.69 (1H, d,  $J$  = 10.0 Hz), 4.98 (1H, dd,  $J$  = 17.4, 1.0 Hz), 4.92 (1H, dd,  $J$  = 10.6, 1.0 Hz), 2  $\times$  1.70 (3H, s), 2  $\times$  1.47 (3H, s).  $^{13}\text{C}$  NMR (151 MHz, chloroform-*d*),  $\delta$  163.7, 160.1, 155.6, 2  $\times$  153.8, 149.5, 141.2, 137.8, 132.4, 131.8, 131.8,

129.9, 126.8, 121.3, 115.6, 112.8, 112.0, 108.5, 2  $\times$  107.2, 77.9, 41.4, 2  $\times$  29.4, 2  $\times$  27.7. DART-MS,  $\text{C}_{26}\text{H}_{24}\text{BrO}_5$  [ $\text{M} + \text{H}$ ] $^+$ ,  $m/z$ : 495.0805 (calcd mass for  $\text{C}_{26}\text{H}_{24}\text{BrO}_5$ ,  $m/z$ : 495.0807),  $\text{C}_{26}\text{H}_{24}^{81}\text{BrO}_5$  [ $\text{M} + \text{H}$ ] $^+$ ,  $m/z$ : 497.0784.

### 3.1.2 Spectral data of compound 1b, 2,2-dimethyl-10-(2-methylbut-3-en-2-yl)-8-oxo-2H,8H-pyrano[3,2-g]chromen-5-yl 3-chlorobenzoate

Nordentatin (107.4 mg, 0.3442 mmol), 3-chlorobenzoyl chloride (90.3 mg, 0.5163 mmol), yield 74.9%,  $\lambda_{\max}$  (log  $\epsilon$ ) 340 (0.772), 268 (1.703), 233 (1.952), 233 (1.952). M.p. 173–174°C. FT-IR 3,080, 2,964, 2,922, 2,852, 1,743, 1,724, 1,620, 1,602, 1,554  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR (chloroform-*d*, 600 MHz)  $\delta$  8.33 (1H, dd,  $J$  = 6.9, 2.1 Hz), 8.16 (1H, ddd,  $J$  = 8.5, 4.5,

2.2 Hz),  $2 \times 7.52$  (1H, d,  $J = 9.7$  Hz), 7.34 (1H, t,  $J = 8.5$  Hz), 6.33 (1H, dd,  $J = 17.4$ , 10.6 Hz), 6.24 (1H, d,  $J = 10.0$  Hz), 6.20 (1H, d,  $J = 9.7$  Hz), 5.71 (1H, d,  $J = 10.0$  Hz), 4.98 (1H, d,  $J = 17.4$  Hz), 4.92 (1H, m), 1.70 (6H, s), 1.48 (6H, s).  $^{13}\text{C}$  NMR data (see the supplementary material). DART-MS,  $\text{C}_{26}\text{H}_{24}\text{ClO}_5$ ,  $m/z$ : 451.1313  $[\text{M} + \text{H}]^+$  (calcd mass for  $\text{C}_{26}\text{H}_{24}\text{ClO}_5$ ,  $m/z$ : 451.1312),  $\text{C}_{26}\text{H}_{24}^{37}\text{ClO}_5$   $[\text{M} + \text{H}]^+$ ,  $m/z$ : 453.1286.

### 3.1.3 Spectral data of compound 1c, 2,2-dimethyl-10-(2-methylbut-3-en-2-yl)-8-oxo-2H,8H-pyrano[3,2-g]chromen-5-yl 2-chloro-4-nitrobenzoate

Nordentatin (115.6 mg, 0.3686 mmol), 2-chloro-4-nitrobenzoyl chloride (121.6 mg, 0.5500 mmol), yield 43.6%. UV (MeOH),  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 340 (0.509), 267 (1.468). M.p. 197–198°C. FT-IR 3,105, 3,088, 2,995, 2,968, 2,933, 1,762, 1,720, 1,618, 1,535  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR (chloroform-*d*, 600 MHz)  $\delta$  8.45 (1H, m), 8.29 (2H, d,  $J = 1.0$  Hz), 7.60 (1H, d,  $J = 9.7$  Hz), 6.32 (2H, m), 6.24 (1H, d,  $J = 9.7$  Hz), 5.76 (1H, d,  $J = 9.7$  Hz), 4.98 (1H, dd,  $J = 17.4$ , 0.9 Hz), 4.92 (1H, dd,  $J = 10.6$ , 0.9 Hz), 1.70 (6H, s), 1.49 (6H, s).  $^{13}\text{C}$  NMR data (see the supplementary material). DART-MS,  $\text{C}_{26}\text{H}_{23}\text{ClNO}_7$ ,  $m/z$ : 496.1162  $[\text{M} + \text{H}]^+$  (calcd mass for  $\text{C}_{26}\text{H}_{23}\text{ClNO}_7$ ,  $m/z$ : 496.1163),  $\text{C}_{26}\text{H}_{23}^{37}\text{ClNO}_7$   $[\text{M} + \text{H}]^+$ ,  $m/z$ : 498.1129.

### 3.1.4 Spectral data of compound 1d, 2,2-dimethyl-10-(2-methylbut-3-en-2-yl)-8-oxo-2H,8H-pyrano[3,2-g]chromen-5-yl 3-chloro-4-fluorobenzoate

Nordentatin (108.2 mg, 0.3467 mmol), 3-chloro-4-fluorobenzoyl chloride (100.3 mg, 0.5201 mmol), yield 54.5%. UV (MeOH),  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 340 (0.772), 268 (1.703), 233 (1.952), 233 (1.952). M.p. 173–174°C. FT-IR 3,086, 3,049, 2,970, 2,935, 2,922, 1,747, 1,720, 1,618, 1,598, 1,548  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR (chloroform-*d*, 600 MHz)  $\delta$  8.33 (1H, dd,  $J = 6.9$ , 2.1 Hz), 8.16 (1H, ddd,  $J = 8.5$ , 4.5, 2.2 Hz), 7.52 (1H, d,  $J = 9.7$  Hz), 7.34 (1H, t,  $J = 8.5$  Hz), 6.33 (1H, dd,  $J = 17.4$ , 10.6 Hz), 6.24 (1H, d,  $J = 10.0$  Hz), 6.20 (1H, d,  $J = 9.7$  Hz), 5.71 (1H, d,  $J = 10.0$  Hz), 4.98 (1H, d,  $J = 17.4$  Hz), 4.94–4.90 (1H, m), 1.70 (6H, s), 1.48 (6H, s).  $^{13}\text{C}$  NMR data (see the supplementary material). DART-MS,  $\text{C}_{26}\text{H}_{23}\text{ClFO}_5$ ,  $m/z$ : 469.1221  $[\text{M} + \text{H}]^+$  (calcd mass for  $\text{C}_{26}\text{H}_{23}\text{ClFO}_5$ ,  $m/z$ : 469.1218),  $\text{C}_{26}\text{H}_{23}^{37}\text{ClFO}_5$   $[\text{M} + \text{H}]^+$ ,  $m/z$ : 471.1191.

### 3.1.5 Spectral data of compound 1e, 2,2-dimethyl-10-(2-methylbut-3-en-2-yl)-8-oxo-2H,8H-pyrano[3,2-g]chromen-5-yl 3-bromobenzoate

Nordentatin (104.8 mg, 0.3358 mmol), 3-bromo-benzoyl chloride (110.5 mg, 0.5037 mmol), yield 93.5%. UV (MeOH),

$\lambda_{\text{max}}$  (log  $\epsilon$ ) 340 (0.782), 268 (1.811), 209 (4.0). M.p. 127–128°C. FT-IR, 3,080, 2,964, 2,935, 2,883, 1,741, 1,724, 1,620, 1,602, 1,554  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR (chloroform-*d*, 600 MHz)  $\delta$  8.32 (1H, dd,  $J = 1.7$  Hz), 8.12 (1H, dt,  $J = 7.8$ , 1.3 Hz), 7.90 (1H, ddd,  $J = 8.0$ , 2.0, 1.0 Hz), 7.54 (1H, d,  $J = 9.7$  Hz), 7.41 (1H, dt,  $J = 7.9$  Hz), 6.33 (1H, dd,  $J = 17.4$ , 10.6 Hz), 6.26 (1H, d,  $J = 10.0$  Hz), 6.21 (1H, d,  $J = 9.7$  Hz), 5.70 (1H, d,  $J = 10.0$  Hz), 4.98 (1H, dd,  $J = 17.4$ , 1.0 Hz), 4.92 (1H, dd,  $J = 10.6$ , 1.0 Hz), 1.70 (6H, s), 1.48 (6H, s).  $^{13}\text{C}$  NMR data (see the supplementary material). DART-MS,  $\text{C}_{26}\text{H}_{24}\text{BrO}_5$ ,  $m/z$ : 495.0802  $[\text{M} + \text{H}]^+$  (calcd mass for  $\text{C}_{26}\text{H}_{24}\text{BrO}_5$ ,  $m/z$ : 495.0807),  $\text{C}_{26}\text{H}_{24}^{81}\text{BrO}_5$   $[\text{M} + \text{H}]^+$ ,  $m/z$ : 497.0779.

### 3.1.6 Spectral data of compound 1f, 2,2-dimethyl-10-(2-methylbut-3-en-2-yl)-8-oxo-2H,8H-pyrano[3,2-g]chromen-5-yl 4-butylbenzoate

Nordentatin (113.6 mg, 0.3467 mmol), 4-butylbenzoyl chloride (107.4 mg, 0.5461 mmol), yield 37%. UV (MeOH),  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 349 (0.79), 257 (2.87), 231 (1.0). M.p. 77–78°C.  $^1\text{H}$  NMR (chloroform-*d*, 600 MHz),  $\delta$   $2 \times 8.16$  (1H, d,  $J = 8.3$  Hz), 7.57 (1H, d,  $J = 9.7$  Hz),  $2 \times 7.37$  (1H, d,  $J = 8.3$  Hz), 6.34 (1H, dd,  $J = 17.4$ , 10.6 Hz), 6.17 (1H, d,  $J = 9.7$  Hz), 5.67 (1H, d,  $J = 10.0$  Hz), 4.98 (1H, dd,  $J = 17.4$ , 1.0 Hz), 4.91 (1H, dd,  $J = 10.6$ , 1.0 Hz), 2.79–2.67 (2H, m), 1.70 (6H, s), 1.67 (4H, tt,  $J = 8.9$ , 7.6 Hz), 1.47 (6H, s), 1.40 (2H, dq,  $J = 14.7$ , 7.4 Hz), 0.96 (3H, t,  $J = 7.4$  Hz).  $^{13}\text{C}$  NMR data (see the supplementary material). DART-MS,  $\text{C}_{30}\text{H}_{33}\text{O}_5$ ,  $m/z$  473.2328  $[\text{M} + \text{H}]^+$  (calcd mass for  $\text{C}_{30}\text{H}_{33}\text{O}_5$ ,  $m/z$ : 473.2328).

### 3.1.7 Spectral data of compound 1g, 2,2-dimethyl-10-(2-methylbut-3-en-2-yl)-8-oxo-2H,8H-pyrano[3,2-g]chromen-5-yl 2,4,6-trichlorobenzoate

Nordentatin (107.6 mg, 0.3448 mmol), 2,4,6-trichlorobenzoyl chloride (126.1 mg, 0.5172 mmol) yield 90%. UV (MeOH),  $\lambda_{\text{max}}$  (log  $\epsilon$ , MeOH), 349 (0.396), 267 (0.949), 259 (0.868). M.p. 149–150°C. FT-IR 3,093, 3,078, 2,999, 2,980, 2,929, 1,762, 1,728, 1,614, 1,600, 1,579, 1,550  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR (chloroform-*d*, 600 MHz),  $\delta$  7.93 (1H, d,  $J = 9.7$  Hz), 7.50 (2H, s), 6.65 (1H, d,  $J = 10.0$  Hz), 6.32 (1H, dd,  $J = 17.4$ , 10.6 Hz), 6.24 (1H, d,  $J = 9.7$  Hz), 5.77 (1H, d,  $J = 10.0$  Hz), 4.97 (1H, dd,  $J = 17.4$ , 0.9 Hz), 4.91 (1H, dd,  $J = 10.6$ , 1.0 Hz), 1.70 (6H, s), 1.49 (6H, s).  $^{13}\text{C}$  NMR data (see the supplementary material). DART-MS,  $\text{C}_{26}\text{H}_{22}\text{Cl}_3\text{O}_5$ ,  $m/z$ : 519.0536  $[\text{M} + \text{H}]^+$  (calcd mass for  $\text{C}_{26}\text{H}_{22}\text{Cl}_3\text{O}_5$ ,  $m/z$ : 519.0533),  $\text{C}_{26}\text{H}_{22}^{37}\text{Cl}_3\text{O}_5$   $[\text{M} + \text{H}]^+$ ,



$m/z$ : 521.0505,  $C_{26}H_{22}^{37}Cl_2ClO_5$   $[M + H]^+$ ,  $m/z$ : 523.0471,  $C_{26}H_{22}^{37}Cl_3O_5$   $[M + H]^+$ ,  $m/z$ : 525.0438.

### 3.1.8 Spectral data of compound 1h, 2,2-dimethyl-10-(2-methylbut-3-en-2-yl)-8-oxo-2H,8H-pyrano[3,2-g]chromen-5-yl 3-(trifluoromethyl)benzoate

Nordentatin (110.7 mg, 0.3548 mmol), 3-(trifluoromethyl)benzoyl chloride (110.9 mg, 0.5322 mmol), yield 65.4%. UV (MeOH),  $\lambda_{max}$  (log  $\epsilon$ ) 352 (1.078), 272 (3.769), 228 (4.0), 209 (2.085). M.p. 77–78°C. FT-IR 3,095, 3,080, 2,966, 2,937, 2,924, 2,883, 1,747, 1,728, 1,620, 1,602, 1,552  $cm^{-1}$ .  $^1H$  NMR (chloroform- $d$ , 600 MHz)  $\delta$  8.51 (1H, s), 8.45 (1H, d,  $J$  = 7.8 Hz), 7.98 (1H, d,  $J$  = 7.9 Hz), 7.74 (1H, t,  $J$  = 7.8 Hz), 7.54 (1H, d,  $J$  = 9.7 Hz), 6.34 (1H, dd,  $J$  = 17.4, 10.6 Hz), 6.26 (1H, d,  $J$  = 10.0 Hz), 6.20 (1H, d,  $J$  = 9.7 Hz), 5.71 (1H, d,  $J$  = 10.0 Hz), 5.02–4.96 (1H, m), 4.95–4.89 (1H, m), 1.71 (6H, s), 1.48 (6H, s).  $^{13}C$  NMR data (see the supplementary material). DART-MS,  $C_{27}H_{24}F_3O_5$ ,  $m/z$ : 485.1571  $[M + H]^+$  (calcd mass for  $C_{27}H_{24}F_3O_5$ ,  $m/z$ : 485.1576).

### 3.1.9 Spectral data of compound 1i, 2,2-dimethyl-10-(2-methylbut-3-en-2-yl)-8-oxo-2H,8H-pyrano[3,2-g]chromen-5-yl 3,5-bis(trifluoromethyl)benzoate

Nordentatin (103.6 mg, 0.33205 mmol), 3,5-bis(trifluoromethyl)benzoyl chloride (121.6 mg, 0.5500 mmol), yield 41%. UV (MeOH),  $\lambda_{max}$  (log  $\epsilon$ ) 349 (0.79), 257 (2.87), 231 (1.0). M.p. 186–187°C. FT-IR 3,089, 3,066, 2,999, 2,981, 2,937, 1,759, 1,737, 1,620, 1,604, 1,552  $cm^{-1}$ .  $^1H$  NMR (chloroform- $d$ , 600 MHz)  $\delta$  8.69 (2H, s), 8.22 (1H, s), 7.51 (1H, d,  $J$  = 9.7 Hz), 6.33 (1H, dd,  $J$  = 17.4, 10.6 Hz), 6.23 (1H, d,  $J$  = 10.0 Hz), 6.21 (1H, d,  $J$  = 9.7 Hz), 5.73 (1H, d,  $J$  = 10.0 Hz), 4.99 (1H, dd,  $J$  = 17.4, 0.8 Hz), 4.93 (1H, dd,  $J$  = 10.6, 0.9 Hz), 1.71 (6H, s), 1.49 (6H, s).  $^{13}C$  NMR data (see the supplementary material). DART-MS  $C_{28}H_{23}F_6O_5$ ,  $m/z$ : 553.1440  $[M + H]^+$  (calcd mass for  $C_{28}H_{23}F_6O_5$ ,  $m/z$ : 553.1450).

### 3.1.10 Spectral data of compound 1j, 2,2-dimethyl-10-(2-methylbut-3-en-2-yl)-8-oxo-2H,8H-pyrano[3,2-g]chromen-5-yl 4-iodobenzoate

Nordentatin (103.0 mg, 0.3301 mmol), 4-iodobenzoyl chloride (131.8 mg, 0.4952 mmol), yield 58%. UV (MeOH),  $\lambda_{max}$  (log  $\epsilon$ ) 349 (0.79), 257 (2.87), 231 (1.0). M.p. 156–157°C. FT-IR 3,072, 2,972, 2,927, 2,870, 1,747,

1,730, 1,643, 1,614, 1,587  $cm^{-1}$ .  $^1H$  NMR (chloroform- $d$ , 600 MHz)  $\delta$  7.95 (4H, s), 7.52 (1H, d,  $J$  = 9.7 Hz), 6.33 (1H, dd,  $J$  = 17.4, 10.6 Hz), 6.25 (1H, d,  $J$  = 10.0 Hz), 6.19 (1H, d,  $J$  = 9.7 Hz), 5.69 (1H, d,  $J$  = 10.0 Hz), 4.98 (1H, dd,  $J$  = 17.4, 0.9 Hz), 4.91 (1H, dd,  $J$  = 10.6, 1.0 Hz), 1.70 (6H, s), 1.47 (6H, s).  $^{13}C$  NMR data (see the supplementary material). DART-MS,  $C_{26}H_{24}IO_5$ ,  $m/z$ : 543.0667  $[M + H]^+$  (calcd mass for  $C_{26}H_{24}IO_5$ ,  $m/z$ : 543.0668).

## 3.2 $\alpha$ -Glucosidase inhibition activity of nordentatin (1) and its derivatives (1a–1j)

All the compounds were evaluated for their inhibitory effect on yeast  $\alpha$ -glucosidase (Table 1). Among the modified compounds, **1b**, **1d**, **1e**, **1f**, **1h**, **1i**, and **1j** exhibited a high inhibition of  $\alpha$ -glucosidase with  $IC_{50}$  values of 1.54, 9.05, 4.87, 20.25, 12.34, 5.67, and 2.43 mM (acarbose 7.57 mM) (Table 1) [23]. The highest inhibition activity was shown when chloride was at the *meta*-position as in **1b**, followed by *para*-substituted iodide (**1j**) and the *meta*-position of bromide (**1e**). They are also comparable with the standard control acarbose. In addition, compounds containing bistrifluoromethane (**1i**), trifluoromethane (**1h**), 3-chloro, 5-fluoro (**1d**), and *para*-butyl groups (**1f**) showed higher activity than nordentatin (**1**) with an  $IC_{50}$  value of 36.7 mM. Based on this study, we can conclude that the activity of some compounds increased after modification, while that of some compounds decreased, e.g., **1a**, **1c**, and **1g** showed no inhibition.

**Table 1:** Results of  $\alpha$ -glucosidase inhibition assay of derivatives **1a–1j**

Compound no.	$IC_{50}$ (mM) Yeast $\alpha$ -glucosidase
<b>1a</b>	NI*
<b>1b</b>	1.54
<b>1c</b>	NI*
<b>1d</b>	9.05
<b>1e</b>	4.87
<b>1f</b>	20.25
<b>1g</b>	NI*
<b>1h</b>	12.34
<b>1i</b>	5.67
<b>1j</b>	2.43
<b>1</b>	36.7
Acarbose (std)	7.57

NI\* = no inhibition at concentration  $\leq 5$  mg/mL

**Table 2:** Investigation of the cytotoxicity of the modified compounds (**1a–1j**) against HeLa and T47D cell lines

Compound name	IC <sub>50</sub> (mM)	
	HeLa	T47D
<b>1a</b>	0.45	0.10
<b>1b</b>	0.39	0.04
<b>1c</b>	0.40	0.24
<b>1d</b>	0.28	0.11
<b>1e</b>	0.59	0.12
<b>1f</b>	1.25	1.57
<b>1g</b>	0.52	0.41
<b>1h</b>	0.34	0.38
<b>1i</b>	0.25	0.24
<b>1j</b>	0.35	0.23
<b>1</b>	0.61	0.04
Doxorubicin	$0.4 \times 10^{-2}$	$0.6 \times 10^{-4}$

### 3.3 Cytotoxic activity of nordentatin (**1**) and modified compounds (**1a–1j**) against HeLa and T47D cell lines

The cytotoxicity of all modified compounds was tested against a HeLa cell line, and their IC<sub>50</sub> values were compared with those of nordentatin (**1**) and the standard control doxorubicin (Table 2). All tested compounds showed low activity with IC<sub>50</sub> values ranging from 0.25 to 1.25 mM against HeLa cells.

However, the results indicated that the modified compounds that contain fluorobenzoyl groups (**1i**, **1d**, **1h**, and **1b**) have more cytotoxic activity than others and nordentatin (**1**). On the other hand, the activity of compounds **1j**, **1c**, **1a**, **1g**, **1e**, and **1f** was lower (Table 2). The investigation of cell proliferation of modified compounds was also performed on a T47D cell line (Table 2). The results revealed that the modified compounds have moderate to low inhibition against the cancer cell line with the IC<sub>50</sub> values ranging from 0.043 to 1.57 mM, but their activity was lower than the activity of the parent compound, nordentatin (**1**) with an IC<sub>50</sub> value of 0.041 mM (Table 2). The structure–activity relationship study showed that the modified compounds with chloro and bromo compounds at *meta* and *para* positions had stronger inhibition than others.

## 4 Conclusions

In the current work, we continue our previous research concerning bioactive secondary metabolites from natural

medicinal plants and their derivatives. As a result, ten new pyranocoumarin benzoate derivatives were semi-synthesized in good to excellent yields from natural nordentatin (**1**) by an acylation method. Both parent and modified compounds were tested for their antidiabetic activity by an  $\alpha$ -glucosidase assay and anticancer activity (HeLa and T47D cell lines) by an MTT assay. Among the tested compounds, modified compound **1b** showed the highest inhibition activity against yeast  $\alpha$ -glucosidase enzymes, in particular 5 times higher than that of acarbose and 20 times higher than that of the parent compound, nordentatin (**1**). Among the tested compounds, modified compound **1i** showed the highest cytotoxic activity against the HeLa cell line. Compound **1b** showed a moderate cytotoxic activity against T47D but it was less effective than the parent compound, nordentatin (**1**). As a result of this study, we can conclude that some modified compounds are promising, since their activity is comparable with that of standard drugs. Especially, active compound **1b**, 2,2-dimethyl-10-(2-methylbut-3-en-2-yl)-8-oxo2*H*,8*H*-pyrano[3,2-*g*]chromen-5-yl-3-chlorobenzoate, should be studied as a potential alternative  $\alpha$ -glucosidase inhibitor.

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