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Hydroxymethylated dihydrocoumarins generated from γ-irradiated esculin exhibit potent α-glucosidase inhibitory effects

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Abstract

Esculin, a coumarin glucoside, was subjected to γ-irradiation in methanolic solution to afford two novel dihydrocoumarin derivatives, esculinosins A (**2**) and B (**3**), via regioselective hydroxymethylation at the *cis*-olefinic bond. The structures of the newly generated compounds **2** and **3** were identified using spectroscopic methods, including nuclear magnetic resonance and mass spectrometry. Compound **2**, bearing two hydroxymethyl functionalities at the C-3 and C-4 positions, exhibited a significantly

enhanced α-glucosidase inhibitory activity (IC_{50} = 36.0 ± 1.0 μM) compared to the parent esculin (IC_{50} = 147.8 ± 2.5 μM). Enzyme kinetic studies confirmed that both compounds function as competitive inhibitors, with esculinosin A (**2**) exhibiting the lowest K_i value (32.1 ± 0.9 μM). These findings suggest that γ-irradiation-induced hydroxymethylation is an effective strategy for improving the biological activities of dihydrocoumarin derivatives, highlighting the potential for developing novel non-sugar-based hypoglycemic agents.

Keywords

esculin; dihydrocoumarin; hydroxymethylation; radiolysis; α-glucosidase inhibitor

Introduction

Coumarins constitute a structurally diverse class of secondary metabolites that are widely distributed in nature, particularly in green plants [1]. Their biosynthesis primarily proceeds through the phenylpropanoid pathway, via shikimic and cinnamic acid intermediates originating from phenylalanine metabolism [2]. Structurally, coumarins share a benzo-*α*-pyrone framework (2*H*-1-benzopyran-2-one) and are classified into subgroups such as simple coumarins, furanocoumarins, and pyranocoumarins [3]. Among them, esculin (esculetin 6-*β*-D-glucoside) is a representative coumarin glucoside primarily found in the bark of *Aesculus hippocastanum* and *Fraxinus rhynchophylla*, a medicinal plant used traditionally for centuries [4,5]. Modern pharmacological studies have demonstrated that esculin possesses various biological activities, including anti-inflammatory, antioxidant, antitumor, anticoagulant, antibacterial, and antidiabetic effects [6]. Furthermore, esculin undergoes metabolic modification by human gut microbiota, producing sulfated and glucosylated derivatives

such as aglycone conjugates [7]. These observations from previous studies indicate that esculin is a promising scaffold for the structural modification and development of bioactive derivatives.

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by sustained hyperglycemia, which contributes to the development of complications such as neuropathy, nephropathy, and cardiovascular disease [8]. Effective management of blood glucose levels, particularly after meals, is essential to prevent these complications. α-Glucosidase (EC 3.2.1.20) is a digestive enzyme located in the small intestinal brush border that catalyzes the final step of carbohydrate hydrolysis, converting oligosaccharides into absorbable monosaccharides [9]. Inhibition of α-glucosidase is a well-established therapeutic approach for delaying glucose absorption and mitigating postprandial glucose spikes. Although synthetic inhibitors such as acarbose are available, their side effects have encouraged the exploration of safer, naturally derived alternatives. Among these, natural coumarins have attracted attention as structurally versatile scaffolds for the development of non-sugar-based hypoglycemic agents.

Recently, ionizing radiation has gained attention as a green and efficient strategy for structural modification of natural compounds [10]. In particular, γ-irradiation in protic solvents such as methanol generates reactive radicals, which can selectively react with electron-deficient moieties like olefinic bonds and carbonyl groups [11,12]. This approach, known as radiation-induced transformation technology (RTT), enables regioselective derivatization under mild, catalyst-free conditions. Compared to conventional chemical or enzymatic methods, RTT offers simplicity, environmental compatibility, and the ability to derivatize structurally complex compounds without

protecting groups [13]. It is particularly valuable for modifying glycosides and phenolics to enhance their solubility, reactivity, or bioactivity [10].

The primary aim of this study was to investigate the effect of γ-irradiation on esculin (**1**), focusing on its structural transformation and the enhancement of its α-glucosidase inhibitory activity, which is directly related to postprandial glucose control in DM [14]. This study highlights the utility of RTT as a tool for generating novel hydroxymethylated coumarin derivatives and underscores its potential in the development of functional agents for metabolic disorders.

Results and Discussion

A solution of esculin (**1**) in methanol was irradiated at doses of 5, 10, 20, and 30 kGy. Among these, the sample irradiated at 30 kGy exhibited the most α-glucosidase inhibitory activity, with an IC_{50} value of 22.9 ± 0.2 μg/mL, which was markedly more potent than that of unirradiated esculin (IC_{50} = 50.2 ± 1.7 μg/mL) (Table S1). To investigate the chemical basis of this enhanced activity, the transformation pattern was analyzed by reversed-phase high performance liquid chromatography (RP-HPLC) (Figure 1). In the chromatogram of the sample irradiated at 30 kGy, esculin (**1**, t_R = 12.2 min) was not detected, suggesting complete conversion. Instead, two predominant peaks were observed at retention times (t_R) of 8.4 (**2**) and 9.7 min (**3**). Based on these results, the 30 kGy-irradiated sample was selected for further purification. Repeated column chromatography of this sample yielded two new hydroxymethylation products, designated compounds **2** and **3** (Figure S1). The structures of these novel dihydrocoumarins were elucidated by interpreting their spectroscopic data (Figure 2).

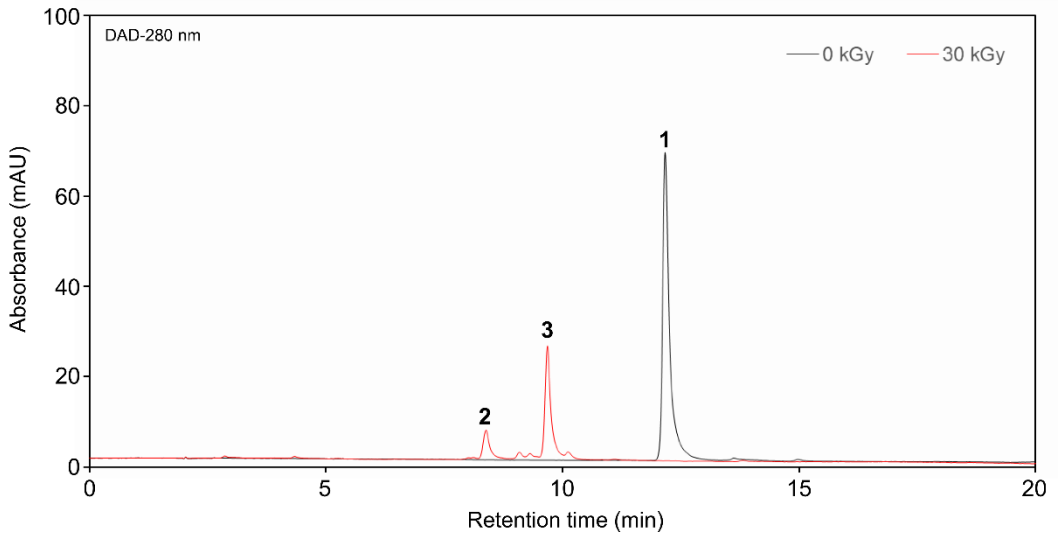

Figure 1: HPLC chromatogram of irradiated esculin mixtures. **1**: esculin, **2**: esculinosin A, **3**: esculinosin B.

Structure elucidation

Esculinosin A (**2**) was obtained as a colorless oil ($[\alpha]_D^{20}$ = −23.6 in MeOH) with the molecular formula $C_{17}H_{22}O_{11}$, as determined by high-resolution electrospray ionization mass spectrometry (HRESIMS) (*m/z* 425.1050 $[M + Na]^+$; calculated for $C_{17}H_{22}O_{11}Na$, 425.1054) and ^{13}C nuclear magnetic resonance (NMR) spectral data showing 17 distinct carbon signals (Table 1). The ultraviolet (UV) spectrum showed characteristic absorptions of a dihydrocoumarin skeleton (λ_{max} 288 nm) [15]. The dihydrocoumarin scaffold in compound **2** was supported by the characteristic signals observed in its ^1H NMR spectrum (Table 1), including two isolated aromatic singlets at δ_H 7.09 (1H, s, H-5) and 6.38 (1H, s, H-8), as well as two methine proton signals at δ_H 3.85 (1H, dd, *J* = 10.2, 3.6 Hz, H-4) and 3.10 (1H, dt, *J* = 10.2, 3.0 Hz, H-3β) [16]. The ^{13}C NMR spectrum further confirmed this scaffold, with characteristic signals at δ_C 180.4 (C-2), 49.9 (C-4), and 39.7 (C-3), consistent with a dihydropyran ring system [16]. In addition, signals corresponding to aromatic quaternary carbons appeared at δ_C 153.5 (C-9), 148.8 (C-7), 139.8 (C-6), and 116.5 (C-10), while the protonated aromatic carbons were assigned at δ_C 120.5 (C-5) and 104.7 (C-8) based on heteronuclear single quantum

coherence (HSQC) correlations. Further evidence for the presence of a glucosidic moiety was provided by an anomeric proton signal at δ_H 4.59 (1H, d, J = 7.8 Hz, H-1'), and multiple oxygenated proton resonances in the 3.89–3.37 (6H, H-2' to H-6') range, typical of monosaccharide protons [17]. Corresponding ^{13}C resonances for the *β*-D-glucopyranosyl unit were observed at δ_C 105.6 (C-1'), 78.4 (C-3'), 77.7 (C-5'), 74.9 (C-2'), 71.4 (C-4'), and 62.5 (C-6'), all consistent with reported values for *β*-glucosides [18]. Two hydroxymethyl substituents were assigned based on the ^1H NMR signals at δ_H 4.54 (1H, t, J = 9.0 Hz, H-11), 4.25 (1H, dd, J = 9.0, 3.6 Hz, H-11), 3.96 (1H, dt, J = 11.4, 3.0 Hz, H-12), and 3.67 (1H, overlap, H-12). The corresponding oxygenated methylene carbon signals for these groups were found at δ_C 72.5 (C-11) and 59.6 (C-12), confirmed through HSQC and correlation spectroscopy (COSY) correlations (Figure 3). These assignments were corroborated by ^{13}C NMR and HSQC spectra, which exhibited high similarity to those of the original esculin [19], except for additional resonances assigned to the newly introduced methine and hydroxymethyl carbons at C-3, C-4, C-11, and C-12.

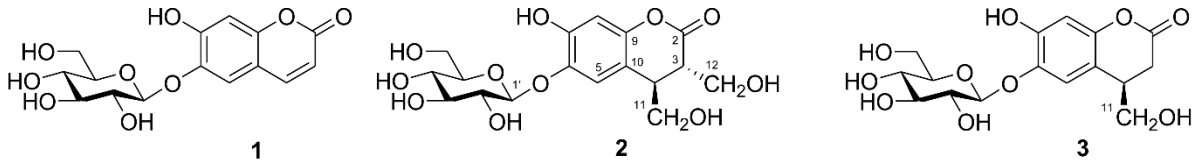

Figure 2: Structures of newly generated dihydrocoumarins **2** and **3** from esculin (**1**) induced by γ radiation.

The attachment of the hydroxymethyl groups at C-3 and C-4 was determined using heteronuclear multiple bond correlation (HMBC) spectral analysis (Figure 3). In particular, the hydroxymethyl functionality H-11 correlated with C-3, 4, and 10, whereas the other hydroxymethyl group H-12 exhibited cross-peaks with C-2, 3, and 4. These well-defined HMBC interactions indicate that the two hydroxymethyl substituents are

located at the C-3 and C-4 positions of the dihydrocoumarin core, confirming the regioselective substitution pattern resulting from γ-radiolysis. In addition, the ^{1}H-^{1}H COSY spectrum supported this structural assignment by revealing sequential scalar couplings between H-12, 2, 3, and 11, consistent with the spatial arrangement inferred from HMBC (Figure 3). Taken together, the HMBC and COSY data provide strong complementary evidence for the connectivity and positioning of the hydroxymethyl groups on the dihydropyran ring, thereby reinforcing the regioselective hydroxymethylation pattern of compound **2**.

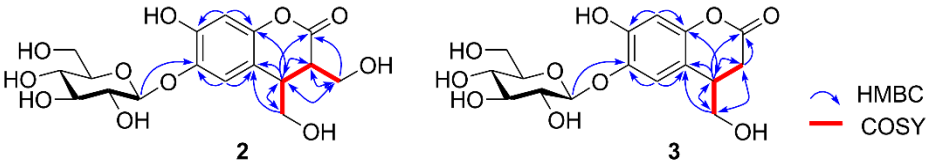

Figure 3: Key HMBC and COSY correlations of dihydrocoumarins **2** and **3**.

The observed nuclear Overhauser effect spectroscopy (NOESY) correlations between H-3β and H-11, as well as between H-4 and H-12, along with the large-coupling constant ($J_{3,4}$ = 10.2 Hz) between H-3β and H-4, strongly support a *threo* configuration for the adjacent stereocenters (Figure 4) [20]. This relative configuration was further supported by the energy-minimized conformation obtained using molecular modeling software (Chem 3D Ultra 10.0), which was consistent with the observed spatial interactions (Figure 4). The glycosidic linkage was assigned as a *β*-glucopyranoside based on the magnitude of the anomeric proton coupling constant (J = 7.8 Hz), which is characteristic of a *β*-configuration in glucopyranosides [17]. Furthermore, the absolute configuration of the sugar unit was unequivocally determined to be D-glucose by HPLC analysis of its phenyl isothiocyanate derivatives using a UV detector, following a previously validated analytical protocol [21]. Based on these spectroscopic

findings, the planar structure of **2** was elucidated and designated esculinosin A, a novel dihydrocoumarin derivative generated via radiolysis (Figure 2).

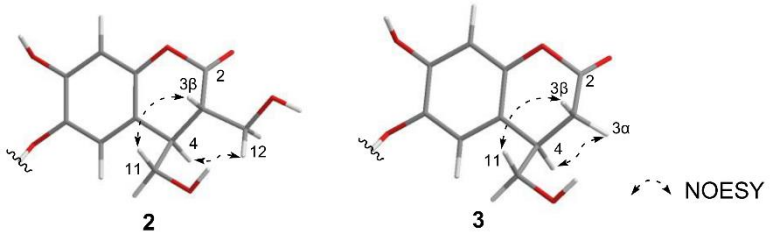

Figure 4: Key NOESY correlations of dihydrocoumarins **2** and **3**.

The molecular formula of compound **3** was determined to be $C_{15}H_{20}O_{10}$ based on HRESIMS data, which showed a quasi-molecular ion peak at *m/z* 395.0941 $[M + Na]^+$ (calculated for $C_{16}H_{20}O_{10}Na$, 395.0948). This compound contains one fewer hydroxymethyl group than compound **2**. The 1D NMR spectral features of **3** closely resembled those of the dihydroxymethylated esculin derivative **2**, with the key distinction being the appearance of a methylene signal at H-3 instead of the hydroxymethyl (H-12) and methine (H-3) resonances observed in compound **2** (Table 1). The position of the remaining hydroxymethyl group (H-11) in **3** was further corroborated by key HMBC correlations from H-11 to C-3, 4, and 10 and from H-3 to C-2, 4, 10, and 11 (Figure 3). The relative stereochemistry at the C-4 position in **3** was elucidated based on NOESY correlations between H-3β and H-11 and between H-3α and H-4 (Figure 4), supporting their spatial proximity and suggesting a fixed 3D arrangement of these protons. Acidic hydrolysis of **3** yielded 4-hydroxymethylated esculetin (**3a**) and *β*-glucopyranose. The stereochemistry at C-4 in **3a** was inferred by comparison with a previously reported levorotatory 4-methyl-dihydrocoumarin with an *R*-configuration at C-4 ($[\alpha]_D$ = +32.0) [22]. The optical rotation value of **3a** ($[\alpha]_D$ = +17.5) suggested an *R*-configuration at the C-4 position. Using the same analytical approach

applied to compound **2**, the relative and absolute configurations of the sugar moiety in **3** were also confirmed to be *β*-D-glucopyranoside [18, 21]. Based on a comprehensive analysis of the 1D and 2D NMR data, the absolute structure of **3** was determined and designated esculinosin B (Figure 2).

Table 1: ^{1}H and ^{13}C NMR shifts[a] of **2** and **3**.

	2		**3**	
Position	δ_H (*J* in Hz)[b]	δ_C, type[c]	δ_H (*J* in Hz)[b]	δ_C, type[c]
2	–	180.4, C	–	180.5, C
3β	3.10 (dt, 10.2, 3.0)	39.7, CH	2.79 (16.8, 2.4)	34.8, CH_2
3α	–	–	2.78 (16.8, 7.2)	
4	3.85 (dd, 10.2, 3.6)	49.9, CH	3.86 (m)	37.6, CH
5	7.09 (s)	120.5, CH	7.04 (s)	119.8, CH
6	–	139.8, C	–	139.7, C
7	–	148.8, C	–	148.6, C
8	6.38 (s)	104.7, CH	6.38 (s)	104.6, CH
9	–	153.5, C	–	153.1, C
10	–	116.5, C	–	117.9, C
11	4.54 (t, 9.0) 4.25 (dd, 9.0, 3.6)	72.5, CH_2	4.62 (t, 9.0) 4.28 (td, 9.0, 3.6)	74.8, CH_2
12	3.96 (dt, 11.4, 3.0) 3.67 (overlap)	59.6, CH_2	–	–
1'	4.59 (d, 7.8)	105.6, CH	4.59 (d, 7.8)	105.8, CH
2'	3.47 (m)	74.9, CH	3.73 (m)	74.9, CH
3'	3.38 (overlap)	78.4, CH	3.39 (m)	78.2, CH
4'	3.37 (overlap)	71.4, CH	3.44 (m)	71.3, CH
5'	3.45 (m)	77.7, CH	3.70 (m)	77.6, CH
6'	3.89 (dd, 12.0, 2.4) 3.66 (overlap)	62.5, CH_2	3.93 (dd, 12.0, 2.4) 3.72 (dd, 12.4, 5.4)	62.4, CH_2

[a]Measured in CD_3OD and assignments of chemical shifts are based on the analysis of 1D- and 2D NMR spectra. The overlapped signals were assigned from HSQC, HMBC,

and ^{1}H-^{1}H COSY spectra without designating multiplicity; [b]Data (δ) measured at 600 MHz; [c]Data (δ) measured at 150 MHz.

γ-Irradiation of methanolic solutions generates various reactive species, including methoxy ($^{\bullet}OCH_3$), hydroxyl ($^{\bullet}OH$), and hydroxyalkyl radicals and hydrogen peroxide (H_2O_2), among which the hydroxymethyl radical ($^{\bullet}CH_2OH$) is the predominant species [23]. Previous studies on flavonoids and phenylpropanoids have shown that hydroxymethyl radicals preferentially react not with aromatic rings, but with electron-deficient sites such as olefinic double bonds or ketone carbonyl groups, leading to selective hydroxymethylation [24, 25]. Notably, γ-irradiation of 4-methylumbelliferone resulted in hydroxymethylation at the C-4 position, affording biologically active dihydrocoumarin derivatives [26]. Consistent with these findings, γ-irradiation of esculin, a coumarin-based glucoside, induced regioselective hydroxymethylation at the *cis*-olefinic bond, yielding hydroxymethylated dihydrocoumarins. Among the two isolated compounds, esculinosin B (**3**) was identified as the major derivative, with a hydroxymethyl substitution at the C-4 position. These observations further supported the regioselective reactivity of hydroxymethyl radicals under radiolytic conditions, particularly at electron-deficient sites within the coumarin framework.

Biological evaluation

Inhibition of α-glucosidase is a well-established therapeutic strategy for managing postprandial hyperglycemia, as it slows carbohydrate digestion and reduces the rate of glucose absorption after meals [9,10]. Therefore, α-glucosidase inhibitory assays are widely used to evaluate the hypoglycemic potential of functional compounds. The two purified esculinosins, A (**2**) and B (**3**), obtained in the current study, were evaluated for their hypoglycemic potential via α-glucosidase inhibition using acarbose as a

positive control [27]. The hydroxymethylated derivatives **2** and **3** of esculin exhibited considerably enhanced inhibitory activity compared to the parent esculin (**1**) (Table 2). Among the two derivatives, esculinosin A (**2**), possessing two hydroxymethyl groups, showed the most potent inhibitory activity against α-glucosidase in a concentration-dependent manner, with an IC_{50} value of 36.0 ± 1.0 μM. Notably, the enhanced inhibitory effect of the hydroxymethylated products correlated with the γ-radiation dose, as the highest activity was observed in the 30-kGy-treated group (Table S1). This finding was consistent with the HPLC analysis (Figure 1), which revealed the dose-dependent formation of novel compounds **2** and **3**, suggesting a close relationship between γ-irradiation-induced structural modification and the observed improvement in hypoglycemic efficacy.

Table 2: Inhibitory activities of hydroxymethylated products **2** and **3** against α-glucosidase.

Compounds	α-Glucosidase IC_{50} (μM)[a]	Inhibition type[b]	K_i (μM)[b]
Esculin (**1**)	147.8 ± 2.5	−	−
2	36.0 ± 1.0	Competitive	32.1 ± 0.9
3	95.1 ± 1.7	Competitive	94.0 ± 1.2
Acarbose[c]	409.7 ± 4.3	−	−

[a]The values indicate the concentration resulting in 50% of the maximum α-glucosidase inhibition. Data are expressed as the mean ± standard deviation of at least four independent experiments; [b]Determined by Lineweaver–Burk plots; [c]Used as a positive control. (−) No test.

As illustrated in Figure 5, kinetic analysis using Lineweaver–Burk plots revealed that both esculinosins A (**2**) and B (**3**) acted as competitive inhibitors of α-glucosidase.

Double-reciprocal plots showed intersecting lines on the *x*-axis, indicating that increasing the substrate concentration overcame this inhibition. The calculated inhibition constants (K_i) were 32.1 ± 0.9 μM for dihydroxymethylated compound **2** and 94.0 ± 1.2 μM for monohydroxymethylated compound **3**. These findings suggest that hydroxymethylation of the *cis*-olefinic region of the coumarin core enhances its inhibitory interaction with α-glucosidase. Furthermore, the degree of hydroxymethylation appears to correlate with α-glucosidase inhibitory potency. Specifically, esculinosin A (**2**), bearing two hydroxymethyl substituents at the C-3 and C-4 positions, exhibited approximately four-fold greater activity than the parent esculin (**1**) and nearly two-fold higher potency than esculinosin B (**3**), which contains only one hydroxymethyl group. This suggests that the introduction of multiple polar functional groups improves the molecular interactions with the active site of the enzyme, thereby enhancing the inhibition efficacy. Notably, both compounds exhibited significantly lower IC_{50} values than the positive control, acarbose, a well-known sugar mimetic inhibitor, indicating that radiolytically derived coumarin analogs could serve as effective non-sugar α-glucosidase inhibitors with a different mechanism of molecular recognition [28]. These findings underscore the potential of γ-irradiation-driven molecular derivatization as a platform for the development of novel hypoglycemic agents derived from natural coumarin glycosides.

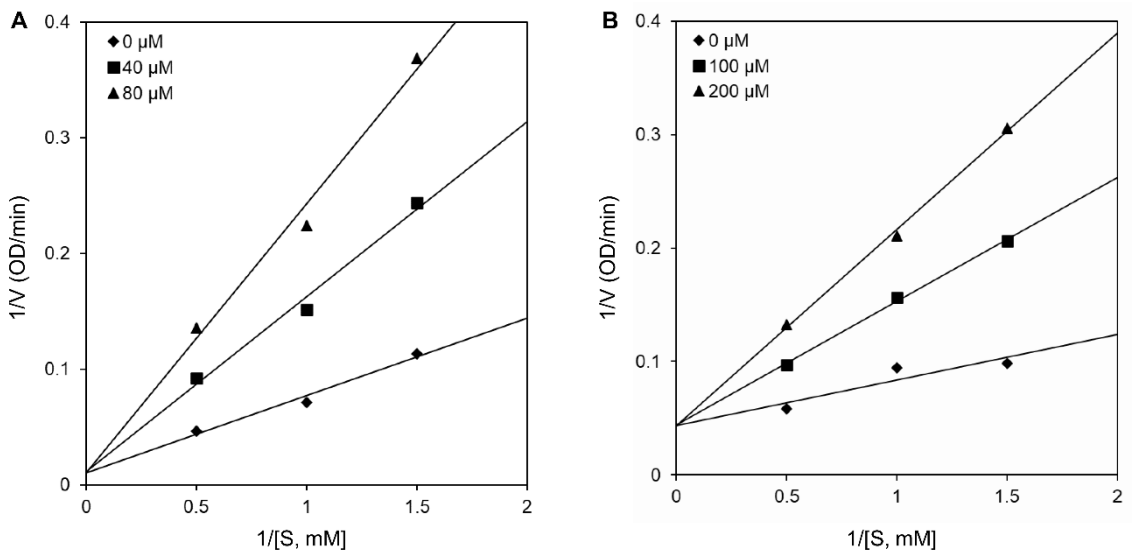

Figure 5: Lineweaver-Burk plots against α-glucosidase inhibition of **2** (**A**) and **3** (**B**).

Conclusion

In summary, γ-irradiation of esculin in methanolic solution led to the discovery of two novel hydroxymethylated dihydrocoumarin derivatives **2** and **3**. Structural elucidation revealed that both compounds were produced through regioselective hydroxymethylation at the *cis*-configured olefinic bond of the coumarin core, mediated by radiolytically generated hydroxymethyl radicals. Notably, esculinosin A (**2**), featuring two hydroxymethyl groups, exhibited a more than two-fold increase in α-glucosidase inhibitory activity compared to esculinosin B (**3**), which contains a single hydroxymethyl functionality. These findings demonstrate that γ-irradiation-induced hydroxymethylation is not only regioselective but also functionally significant, contributing directly to the enhancement of bioactivity. This study underscores the utility of radiation-induced molecular transformation as a powerful approach for the discovery of novel α-glucosidase inhibitors and the development of non-sugar-based hypoglycemic agents. Future research should expand this strategy to achieve broader

structural diversification of natural glycosides and generate functionally optimized lead compounds for the treatment of metabolic disorders.

Experimental

Chemicals and instruments

Esculin, acetonitrile, methanol, formic acid (HPLC grade), deuterated methanol, α-glucosidase (EC 3.2.1.20) from *Saccharomyces cerevisiae*, and *p*-nitrophenyl-*α*-D-glucopyranoside (*p*-NPG) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other reagents and chemicals used in this study were of analytical grade. Spectra of ^{1}H and ^{13}C NMR were acquired using an Avance NEO-600 instrument (Bruker, Karlsruhe, Germany) operated at 600 and 150 MHz, respectively. Chemical shifts are given in ppm in CD_3OD (δ_H 3.05; δ_C 49.0) relative to a tetramethylsilane reference. ESI mass spectra were obtained using a Vanquish UPLC System (Thermo Fisher Scientific, MA, USA). The UV spectrum was measured using a T-60 spectrophotometer (PG Instruments, Leicestershire, UK), and the optical rotation was recorded using a JASCO P-2000 spectrometer (JASCO, Tokyo, Japan). Column chromatography was performed using Toyopearl HW-40 (coarse grade; Tosoh Co., Tokyo, Japan) and YMC gel ODS AQ 120-50S (particle size 50 μm; YMC Co., Kyoto, Japan) gel columns, and a microplate reader (Infinite F200; Tecan Austria GmbH, Grodig, Austria) was used to measure the absorbance. A semi-preparative high-performance liquid chromatography (HPLC) system (Agilent HPLC 1200, Agilent Technologies, Palo Alto, CA, USA) equipped with a photodiode array detector (PDA, 1200 Infinity series, Agilent Technologies) and a series of YMC-Pack ODS A-302 columns (4.6 mm i.d. × 150 mm, particle size 5 μm; YMC Co., Kyoto, Japan) were used to purify the compounds.

γ-Irradiation

Ionizing radiation was carried out at 24 °C, using a cobalt-60 irradiator (Point source AELC, IR-79, MDS Nordion International Co. Ltd.) at the Advanced Radiation Technology Institute, Korea Atomic Energy Research Institute (Jeongup, Korea). The source strength was approximately 320 kCi, with a dose rate of 10 kGy/h. Pure esculin (500 mg) in methanol (500 mL) in a closed glass bottle was directly irradiated at doses of 5, 10, 20, and 30 kGy. The methanolic solution of each irradiated sample was immediately evaporated to remove the solvent and dried. Of the resultant samples, a solution containing esculin irradiated at a dose of 30 kGy exhibited the most enhanced hypoglycemic activities in α-glucosidase inhibition assays (Table S1).

Isolation

The most active 30-kGy-irradiated sample (450 mg) was subjected to flash column chromatography on a Toyopearl HW-40 column using a gradient of H_2O–MeOH (100:0 to 60:40) followed by 70% aqueous acetone. This process yielded four subfractions, designated IREC01 to IREC04. Subfraction IREC02 (144.5 mg), corresponding to the 20% MeOH eluate, was purified via reversed-phase column chromatography on a YMC gel ODS AQ 120-50S column (1.5 cm i.d. × 43 cm) using aqueous methanol to afford compound **3** (57.8 mg, t_R = 9.7 min). Similarly, subfraction IREC03 (70.1 mg) obtained from the 15% MeOH eluate was further purified using the same column (1.5 cm i.d. × 38 cm) to isolate compound **2** (8.0 mg, t_R = 8.3 min).

Esculinosin A (**2**): Colorless oil; $[\alpha]^{20}_D$ –23.6 (*c* 0.1, MeOH); UV λ_{max} MeOH nm (log ε): 205 (3.50), 288 (1.05) nm; ^{1}H and ^{13}C NMR data (Table 1); ESIMS *m/z* 425 $[M + Na]^+$; HRESIMS *m/z* 425.1050 $[M + Na]^+$ (calculated for $C_{17}H_{22}O_{11}Na$, 425.1054) (Figures S2-8).

Esculinosin B (**3**): White amorphous powder; $[\alpha]^{20}_{D}$ +41.5 (*c* 0.1, MeOH); UV λ_{max} MeOH nm (log ε): 206 (3.57), 288 (1.09) nm; ^{1}H and ^{13}C NMR data (Table 1); ESIMS *m/z* 395 $[M + Na]^+$; HRESIMS *m/z* 395.0941 $[M + Na]^+$ (calculated for $C_{16}H_{20}O_{10}Na$, 395.0948) (Figures S9-15).

Determination of the absolute configurations of sugars

Compounds **2** and **3** (1.3 mg each) were hydrolyzed with 0.5 N hydrochloric acid (1 mL) at 70 °C for 2 h to cleave the glycosidic bond. After hydrolysis, the mixtures were extracted with ethyl acetate to separate the aglycone fractions. The aqueous layers containing the liberated monosaccharides were derivatized to determine their absolute configurations. Each solution was treated with L-cysteine methyl ester hydrochloride (1.5 mg) in pyridine (0.5 mL) and incubated at 70°C for 1 h. Subsequently, *o*-tolyl isothiocyanate (100 µL) was added, and the reaction was continued for an additional 1 h at the same temperature. Standard D- and L-glucose samples were derivatized under identical conditions for comparison [20]. The resulting thiocarbamoyl-thiazolidine derivatives were analyzed by HPLC on a YMC Triart C_{18} column (4.6 mm i.d. × 250 mm, 5 µm particle size) at 40°C with isocratic elution using a mixture of H_2O and acetonitrile (75:25, v/v) at a flow rate of 0.8 mL/min. UV detection was performed at 254 nm. The t_R of the derivatives of compounds **2** and **3** were both 16.7 min, which is consistent with that of the D-glucose standard. By contrast, the L-glucose derivative eluted at 15.5 min (Figure S16).

α-Glucosidase inhibition assay

Assay of α-glucosidase inhibitory activity: A previously reported method with a minor modification was used for the evaluation of the ability of the compounds to inhibit α-glycosidase [27]. Briefly, α-glucosidase (10 µL) was incubated in 100 mM potassium

phosphate buffer (pH 6.8). The sample solution (10 µL) was premixed with 90 µL of 100 mM potassium phosphate buffer (pH 6.8). After incubation at 37.5 °C for 30 min, *p*-NPG (1mM substrate) was added to initiate the reaction. The reaction mixture was incubated at 37.5 °C for 30 min, and 100 µL of 2 M NaOH was then added to stop the reaction. The amount of *p*-nitrophenol released was measured at 410 nm using a UV microplate reader (Infinite F200; Tecan Austria GmbH, Grödig, Austria). The half-maximal inhibitory concentration (IC_{50}) was calculated by linear regression analysis of the activity under the assay conditions. Acarbose was used as a positive control, and all assays were carried out in triplicate. Kinetic parameters were determined using a Lineweaver–Burk double-reciprocal plot at increasing concentrations of the substrate and inhibitor. The data were analyzed using Sigma Plot (SPCC Inc., IL, USA), a nonlinear regression program.

Statistical analysis

All experiments were performed in triplicate. Data for *in vitro* analyses of α-glucosidase inhibitory activities were analyzed using the Proc GLM procedure in SAS (version 9.3, SAS Institute Inc., Cary, NC, USA). The results are reported as the least square mean values and standard deviations. Statistical significance was considered at $P < 0.05$.

Supporting Information

Supporting Information File 1: HPLC chromatograms, NMR spectra, and HRESIMS data for new compounds **2** and **3.**

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Competing Interest

The authors declare no competing interests

Author Contributions

Gyeong Han Jeong: conceptualization, methodology, investigation, resources, writing—original draft, writing—review and editing. Hanui Lee: conceptualization, methodology, formal analysis, investigation, writing—original draft. Seung Ae Kim: conceptualization, methodology. Byung Yeoup Chung: supervision, resources, writing—review and editing. Tae Hoon Kim: investigation, writing—original draft, writing—review and editing. Hyoung-Woo Bai: project administration, validation, visualization, writing—review and editing.

Data availability

The datasets used and/or analyzed in the current study are available from the corresponding author upon reasonable request.

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