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Nonthermal plasma-induced degradation of morin and enhancement of biological activities

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Abstract: In the present study, non-thermal dielectric barrier discharge (DBD) plasma of induced structural changes of morin resulted in the isolation of previously undescribed benzofuranone derivative **1**, along with known compounds **2** and **3**. The chemical structures of these degradation products were elucidated by UV, NMR and FABMS spectroscopic analyses. The isolated compounds **1**−**3** showed potent antioxidative activities in two different tests, with IC_{50} values in the range 12.9−41.8 μM in the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^+$) radical scavenging activity, 19.0−71.9 μM for hydroxyl radical scavenging activity test. Furthermore, the new methoxylated benzofuranone **1** exhibited enhancement of inhibitory effects against pancreatic lipase with an IC_{50} value of 90.7±1.6 μM, when compared to the parent morin. These results suggested that the degradation products isolated from plasma exposed morin might be beneficial for prevention of obesity and related diseases.

Keywords: morin • plasma treatment • degradation • radical scavenging • pancreatic lipase

Introduction

Reactive oxygen species (ROS) and free radicals include numerous reactive molecules derived from oxygen, such as hydroxyl radical (OH·), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), and superoxide anion radical (O_2^-).[1, 2] Excessive levels of ROS and free radicals produced by human cells play a key role in the initiation of major disorders, including cardiovascular disease, cancer, aging, inflammation, and toxicity.[3–5] Previously used synthetic antioxidants are butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) and *in vitro* and *in vivo* tests have indicated them to be carcinogenic and affect lipid metabolism disorder.[6] Several safe antioxidant are FDA approved drugs including Edaravone and others and the development of more effective and safe antioxidants is desirable.[7]

Obesity is caused by an imbalance between excessive energy intake and low energy consumption. Excess energy accumulates in fat cells, resulting in their increase and subsequent obesity, and is a strong risk factor for a variety of diseases, including type II diabetes, arteriosclerosis, hypertension, and hyperlipidemia.[8] These metabolic diseases are caused by oxidative stress in the body and imbalances in the antioxidant system.[9] Pancreatic lipase is secreted from the pancreas, and is a key enzyme involved in the digestion and absorption of triglycerides, involved in the hydrolysis of triglycerides into 2-monoglycerides and fatty acids.[10] Thus, the inhibitory activity of pancreatic lipase is an effective strategy for treating obesity in humans. Representative pancreatic lipase inhibitors include orlistat isolated from *Streptomyces toxytrinini*, but adverse side effects have been reported.[11] Therefore, researches have shifted their focus for the development of novel enzyme inhibitors from natural sources.[12]

Flavonoids are naturally occurring phenolic compounds distributed in various foods including fruits, vegetables, and juices. Also, dietary flavonoids are well-known secondary metabolites of plants exerting significant biological effects.[13] Morin is a typical natural yellow flavonol, and is found in fruits of *Maclura pomifera* (osage orange)[14] and leaves of *Psidium guajava* (common guava).[15] Morin is known to possess a diverse spectrum of biological properties including antioxidant, anti-diabetic, antifungal, and antitumor.[16-19]

Dielectric barrier discharge (DBD) plasma treatment is used for non-thermal sterilization method in food processing and is known to exert various physiological functions, including sterilization effects and enhancement of biological activity.[20, 21] However, research on changes in chemical structure and the newly generated products of biological activities using plasma treatment are very limited. As part of an ongoing investigation for generating bioactive compounds using thermal treatment, Fe-catalyzed oxidation, and gamma-irradiation,[22–25] we report here the degradation of morin using DBD plasma, with the consequent formation of a new methoxylated benzofuranone **1**, along with two known compounds **2** and **3**. The newly generated moriplasmin (**1**) shows significantly enhanced antioxidant and anti-obesity effects relative to the parent morin.

Results and Discussion

Structure determination of new degradation product

The morin dissolved in CH_3OH was treated with DBD plasma for 60 min, and the degradation product were monitored by HPLC analysis. Repeated column chromatographic separation of the 60 min reactant led to isolation of the new degraded product **1**, and two known compounds **2** and **3**. The structural characteristics of the new compound are detailed below.

Compound **1** was purified as a yellow amorphous powder with a negative optical rotation ($[\alpha]^{20}_D$ −6.7°, in MeOH). Its HRFABMS data exhibited a hydrogenated ion peak at *m/z* 333.0611 $[M+H]^+$, establishing the molecular formula as $C_{16}H_{13}O_8$. The UV spectrum (λ_{max} 295) showed characteristic

absorption of the benzofuranone skeleton.[26] The ^{1}H-NMR spectrum of **1** exhibited ABX type aromatic protons signals at δ_H 7.32 (1H, d, J = 8.4 Hz, H-6'), 6.30 (1H, dd, J = 8.4, 1.8 Hz, H-5'), and 6.21 (1H, d, J = 1.8 Hz, H-3'), and *meta*-coupled AB type signals at δ_H 5.94 (1H, d, J = 1.2 Hz, H-6) and 5.90 (1H, d, J = 1.2 Hz, H-8). Also, compound **1** showed resonances corresponding to methoxy singlet at δ_H 3.02 (3H, s, 2-OCH_3).[27] The 16 carbon resonances in the ^{13}C-NMR and HSQC spectra were characterized as two ketone signals at δ_C 191.8 (C-4) and 187.9 (C-3), a doubly oxygenated carbon signal at δ_C 109.0 (C-2), and a methoxyl group at 51.8 (2-OCH_3). The methoxyl group was located at the C-2 position and supported by the key HMBC correlation between 2-OCH_3 to C-2 (**Figure. 1**). Consistent with these 1D- and 2D-NMR spectra, compound **1** closely resembled 2-(2,4-dihydroxybenzoyl)-2,4,6-trihydroxy-3(2*H*)-benzofuranone (**2**).[28] However, compound **1** differed from **2** only at position C-2, where the hydroxyl group was replaced with a methoxyl group.

The absolute structure could not be determined due to the apparent unavailability of a relevant reference CD or specific optical rotation value in literature. Therefore, new methoxylated benzofuranone **1** was assigned the trivial name moriplasmin (**Figure. 1**). The isolated known compounds were identified as 2-(2,4-dihydroxybenzoyl)-2,4,6-trihydroxy-3(2*H*)-benzofuranone (**2**) [28, 29] and β-resorcylic acid (**3**)[30, 31] by comparison of the spectroscopic data with available literature values.

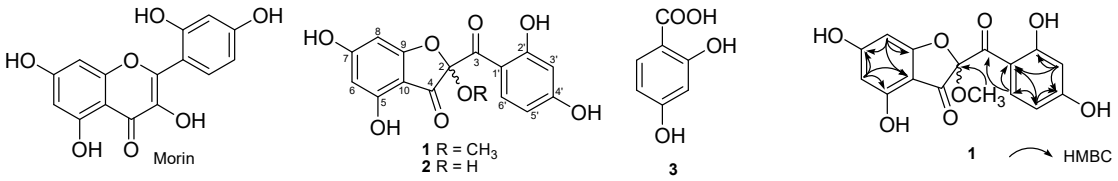

Figure 1. Chemical structures of compounds **1**–**3** isolated from plasma treatment of morin and key HMBC correlations for moriplasmin (**1**).

Radical scavenging activity of degraded products

The antioxidant activity of the newly generated products **1**−**3** was evaluated spectrophotometrically (**Table 1**). The 60 min plasma treated morin showed great improvement in the antioxidant ($ABTS^+$ and hydroxyl radical scavenging) activities, with IC_{50} values of 27.6±1.3 and 40.4±1.6 μg/mL, respectively, as compared to the original morin (IC_{50}: >300 μM). Among the isolated compounds from plasma treated morin, moriplasmin (**1**) exhibited the most potent $ABTS^+$ and hydroxyl radical scavenging activities, with IC_{50} values of 12.9±0.9 and 19.0±1.7 μM, respectively. In the radical scavenging activity tests, moriplasmin (**1**), with a methoxy moiety at C-2, showed stronger efficacy than compound **2** (with a hydroxyl moiety at C-2) (**Figure 1** and **Table 1**). The simple phenolic acid β-resorcylic acid (**3**) showed potent $ABTS^+$ and hydroxyl radical scavenging activities, with IC_{50} values of 41.8±1.5 and 71.9±2.7 μM, respectively, when compared to the morin (**Table 1**). These newly generated products might therefore contribute to the potent radical scavenging activities of the 60 min plasma treated morin, which can be explained by the presence of the benzofuranone derivative.[32, 33]

Table 1. Radical scavenging and pancreatic lipase inhibitory activities of plasma treated morin and degraded products **1**–**3**.

Compound	IC50 value (μM)[a]		
	$ABTS^+$	hydroxyl	Pancreatic lipase
Morin	>300	>300	>500
Plasma treated morin (20 min)	82.3±2.3[b]	69.2±2.4[b]	>500[b]
Plasma treated morin (40 min)	52.4±2.0[b]	58.9±1.7[b]	380.2±4.1[b]
Plasma treated morin (60 min)	27.6±1.3[b]	40.4±1.6[b]	220.3±3.7[b]
1	12.9±0.9	19.0±1.7	90.7±1.6
2	19.4±1.1	44.1±2.6	124.9±2.2
3	41.8±1.5	71.9±2.7	429.7±9.4
(+)-Catechin[c]	17.9±0.9	224.3±4.3	–
Orlistat[c]	–	–	0.8±0.1

[a] All compounds were examined in triplicate experiments. [b] Results expressed as IC_{50} values using μg/mL. [c] Used as a positive control

Pancreatic lipase inhibitory effect of degraded products

The pancreatic lipase inhibitory effect of the plasma treated morin enhanced in a time-dependent manner up to 60 min (IC_{50}: 220.3±3.7 μg/mL) exposure of DBD plasma, in comparison with that of pure morin (IC_{50}: >500 μM). As summarized in **Table 1**, the degradation product **1** secreted from plasma treated morin has a methoxy group at the C-2 position in the benzofuranone skeleton, and shows significantly potent inhibitory effect against

pancreatic lipase (IC_{50} 90.7±1.6 μM). Also, hydroxylation at the C-2 location of benzofuranone derivative **2** (IC_{50}: 124.9±2.2 μM) was found to exhibit relatively weaker inhibitory activity than compound **1**, whereas β-resorcylic acid (**3**) exhibited slightly enhanced pancreatic lipase inhibitory effects (IC_{50} 429.7±9.4 μM). The pancreatic lipase inhibition test shows a similar pattern for radical scavenging activities (**Table 1**).

Recently, Jeong et al.[34] reported a very interesting phenomenon concerning the pancreatic lipase inhibitory effect of phloridzin. They reported that the dimerized phloridzin, which was produced from DBD plasma treatment, imparts a strong effect. Furthermore, the benzofuranone derivative (±)-alphitonin was isolated from the DBD plasma treated quercetin, and exerted enhanced DPPH radical scavenging and α-glucosidase inhibitory effect.[32] To the best of our knowledge, improvement in the anti-obesity of plasma treated morin may therefore be attributed to plasma processing and resultant degradation of morin.

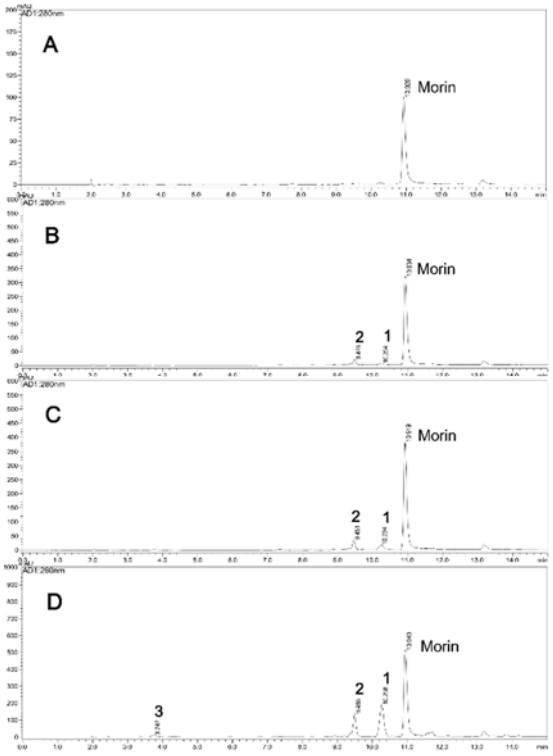

Figure 2. HPLC chromatograms of plasma treated morin for three different times: (**A**) 0, (**B**) 20, (**C**) 40 min, and (**D**) 60 min. See the experimental section for anylsis conditions. **1**: moriplasmin, **2**: 2-(2,4-dihydroxybenzoyl)-2,4,6-trihydroxybenzofuran-3(2H)-one, **3**: β-resorcylic acid.

Quantitative analysis of degraded products

Absolute contents of compounds **1**–**3** isolated from plasma treated morin at 20, 40, and 60 min was quantified by HPLC using a calibration curve (**Figure. 2** and **Table 2**). To further evaluate the relationship between the pancreatic lipase inhibitory activity and the composition of the degraded mixture, the active product contents were identified by HPLC analysis using a PDA detector. Retention times of the newly generated moriplasmin (**1**) (t_R 10.2 min), 2-(2,4-dihydroxybenzoyl)-2,4,6-trihydroxy-3(2*H*)-benzofuranone (**2**) (t_R 9.5 min), and β-resorcylic acid (**3**) (t_R 3.7 min) were detected by plasma treated morin (t_R 10.9 min) at three different time points. HPLC analysis reveals that the amount of the most potent active compound **1** in the plasma treated morin at 20, 40, and 60 min was 8.1±0.2, 31.3±0.8, and 162.3±0.8 mg/g, respectively (**Table 2**), which is in accordance with the enhanced radical scavenging and pancreatic lipase inhibitory activity of each degraded mixture. Previously reported studies suggested that ethane is conversable to the methane during the DBD plasma treatment and also indicated methoxylation of hydroxyl group in the molecules. [35, 36] Therefore, the change pattern similarity of HPLC chromatograms of methanolic and ethanolic solutions containing morin predictable. Hence, other acetone and aqueous conditions under plasma exposure is could not detected methoxylated compound **1**. Further systematic investigation of degraded mechanism under different solvent condition of natural products will be performed.

Table 2. Content (mg/g) of individual components in the degraded mixtures.

		Plasma treatment time (min) [a]		
Compounds	**t_R (min)**	**20**	**40**	**60**
1	10.2	8.1±0.2	31.3±0.8	162.3±0.8

2	9.5	11.9±0.3	30.1±1.2	145.7±0.7
3	3.7	nd [b]	nd [b]	32.1±0.9
Morin	10.9	923.6±1.1	820.0±1.0	529.3±0.9

[a] Different letters within the same row indicate significant differences ($p < 0.05$). [b] nd: Not detected.

Conclusions

When the structures of the degraded products medicated by plasma treatment for 60 min were investigated and new benzofuranone derivative, moriplasmin (**1**) as well as two known related compounds 2-(2,4-dihydroxybenzoyl)-2,4,6-trihydroxy-3(2H)-benzofuranone (**2**) and β-resorcylic acid (**3**) was identified. Their structures of the degradation products were established by detailed 1D-, and 2D-NMR experiment as well as FABMS data. Generation of morinplasmin (**1**) significantly increased up to 162.3±0.8 mg/g in the 60 min treatment. The new methoxylated benzofuranone **1** exhibited most potent radicals scavenging and pancreatic lipase inhibitory activity than the parent morin. The degraded products of morin by DBD plasma treatment may be a possibility therapeutic agent for treatment of antioxidant, anti-obesity and related diseases.

When the structures of the degraded products induced by plasma treatment for 60 min were investigated, a new benzofuranone derivative, moriplasmin (**1**), as well as two known related compounds, 2-(2,4-dihydroxybenzoyl)-2,4,6-trihydroxy-3(2H)-benzofuranone (**2**) and β-resorcylic acid (**3**), were identified. Structures of the degradation products were established by detailed 1D- and 2D-NMR experiments as well as FABMS data. Generation of morinplasmin (**1**) was significantly increased (162.3±0.8 mg/g) in the 60 min treatment. The new methoxylated benzofuranone **1** exerted the maximum potency for radical scavenging and pancreatic lipase inhibitory activities, as compared to the parent morin. We propose that the degraded products of morin by DBD plasma treatment is a potential therapeutic agent for treating antioxidant, anti-obesity, and related diseases.

Experimental Section

General information

Morin (≥98%), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^+$), deoxyribose, (+)-catechin, orlistat, methanol (CH_3OH), acetonitrile (CH_3CN), formic acid (HCOOH), pancreatic lipase from porcine, and *p*-nitrophenyl-butyrate (*p*-NPB) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other materials used in bioassays and analyses were of analytical grade. The optical rotation was obtained using a JASCO P-2000 polarimeter (JASCO, Tokyo, Japan), and the ultraviolet (UV) spectrum was measured on a T-60 spectrophotometer (PG Instrument, Leicestershire, UK). ^{1}H-, ^{13}C-NMR, HSQC, and HMBC spectra were recorded on Bruker Avance DRX-600 MHz spectrometer equipment (Bruker, Billerica, MA, USA) using CD_3OD (δ_H 3.35, δ_C 49.0) as solvent and tetramethysilane (TMS) as internal standard. Fast atom bombardment mass spectrometer (FABMS) was measured on a JMS-700 GC-HRMS spectrometer (JEOL, Tokyo, Japan).

Preparation of treated sample

The dielectric barrier discharge (DBD) instrument used was previously installed on the inner walls of a parallelepiped plastic container (20 × 24 × 10 cm).[35] The actuator was made of polytetrafluoroethylene sheet and copper electrodes. A bipolar square-waveform voltage at 2.5 kHz was applied to the plasma electrode, while the other electrode was grounded. The DBD plasma was generated in the interior of the container with an input frequency of 4.0 Vpp at 25°C. Standard morin solution in CH_3OH (2 L; 200 μg/mL) was placed in a glass dish at the bottom of the container, and treated with DBD plasma for 20, 40, and 60 min. The solvent was removed immediately by evaporation from the plasma treated reactant.

Determination and isolation of degradation products

Reversed-phase high performance liquid chromatography (HPLC) (Shimadzu, Tokyo, Japan) with a PDA detector was used for the chromatographic separation of the degraded products of morin by DBD plasma treatment.[36] HPLC analysis was performed using the YMC-Pack ODS A-302 column (YMC Co., Kyoto, Japan), and the mobile phase consisted of a linear gradient that commenced with 0.1% HCOOH in H_2O, and increased to CH_3CN over 15 min (detection: UV 280 nm; flow rate: 1.0 mL/min; oven temperature: 40°C). The degradation products from morin were identified using their retention times (t_R) and compared with original morin. Quantitative analysis of the isolated compounds was carried out by HPLC using the external standard method by composing standard curves.[32]

A sample solution containing morin was directly treated for 20, 40, and 60 min using DBD plasma, and the converted degradation mixtures were analyzed by HPLC. Among the dried reactants, the morin sample treated for 60 min showed the most enhanced $ABTS^+$, hydroxyl radical scavenging, and pancreatic lipase inhibitory effects (IC_{50} 22.6±1.6, 40.4±1.6, and 220.3±3.7 μg/mL, respectively) (**Table 1**). The solution containing morin that was plasma treated for 60 min was evaporated and suspended in 10% CH_3OH in H_2O (50 mL); the resultant solution was partitioned with ethyl acetate (EtOAc) (3 × 50 mL) to provide a dried EtOAc soluble layer (352.3 mg). A part of the EtOAc soluble layer (332.3 mg) was subjected to column chromatography on an ODS AQ 120-50S gel column (1.0 cm i.d. × 38 cm) with H_2O in a stepwise gradient system containing increasing amounts of CH_3OH. The 15% CH_3OH solvent eluted to yield pure compound **3** (3.5 mg, t_R 3.7 min), 35% CH_3OH solvent eluted to yield pure **2** (17.0 mg, t_R 9.5 min), 40% CH_3OH solvent eluted to yield pure **1** (32.1 mg, t_R 10.3 min), and 50% CH_3OH solvent eluted to yield morin (128.0 mg, t_R 10.9 min) (**Figures S6**). Chemical structures of the newly generated products **1**–**3** were elucidated by 1D-, 2D-NMR, and FABMS spectroscopic analyses (**Figure 1**).

Moriplasmin (**1**): yellow amorphous power, $[\alpha]^{20}_D$ −6.7° (*c* 0.1, MeOH); UV λ_{max} CH_3OH (log ε): 205 (4.20), 231 (3.95), 295 (3.26) nm; FABMS *m/z* 333 $[M+H]^+$, HRFABMS *m/z* 333.0611 $[M+H]^+$ (calcd. for $C_{16}H_{13}O_8$, 333.0610); ^1H NMR (CD_3OD, 600 MHz): δ 7.32 (1H, d, *J* = 8.4 Hz, H-6'), 6.30 (1H, dd, *J* = 8.4, 1.8 Hz, H-5'), 6.21 (1H, d, *J* = 1.8 Hz, H-3'), 5.94 (1H, d, *J* = 1.2 Hz, H-6), 5.90 (1H, d, *J* = 1.2 Hz, H-8), 3.02 (3H, s, 2-OCH_3); ^{13}C NMR (CD_3OD, 150 MHz), δ 191.8 (C-4), 187.9 (C-3), 168.8 (C-9), 164.3 (C-5), 160.7 (C-7), 159.3 (C-2'), 156.2 (C-4'), 131.6 (C-6'), 109.9 (C-5'), 109.7 (C-1'), 109.0 (C-2), 106.7 (C-3'), 103.8 (C-10), 96.5 (C-6), 95.7 (C-8), 51.8 (2-OCH_3) (**Figure. S1-S5**).

2-(2,4-dihydroxybenzoyl)-2,4,6-trihydroxy-3(2H)-benzofuranone (**2**): yellow amorphous powder, $[\alpha]^{20}_D$ −12.7° (*c* 0.1, MeOH); FABMS *m/z* 319 $[M+H]^+$, ^1H NMR (CD_3OD, 600 MHz): δ 8.10 (1H, d, *J* = 8.4 Hz, H-6'), 6.23 (1H, dd, *J* = 8.4, 1.8 Hz, H-5'), 6.17 (1H, d, *J* = 1.8 Hz, H-3'), 5.87 (1H, d, *J* = 1.2 Hz, H-6), 5.84 (1H, d, *J* = 1.2 Hz, H-8); ^{13}C NMR (CD_3OD, 150 MHz), δ 194.2 (C-3), 191.4 (C-4), 172.4 (C-9), 169.9 (C-7), 166.8 (C-4'), 165.8 (C-2'), 159.1 (C-5), 135.1 (C-6'), 109.8 (C-1'), 108.2 (C-5'), 104.5 (C-2), 103.9 (C-3'), 101.9 (C-10), 96.5 (C-6), 90.6 (C-8).[28, 29]

β-resorcylic acid (**3**): white amorphous powder, FABMS *m/z* 155 $[M+H]^+$, ^1H NMR (CD_3OD, 600 MHz): δ 7.50 (1H, d, *J* = 8.4 Hz, H-6), 6.27 (1H, dd, *J* = 8.4, 1.8 Hz, H-5), 6.19 (1H, d, *J* = 1.8 Hz, H-3).[30, 31]

Evaluation of $ABTS^+$ and hydroxyl radical scavenging activity

$ABTS^+$ radical was prepared by reacting 5 mL $ABTS^+$ (7 mM) ethanol (EtOH) solution with 5 mL 2.4 mM potassium persulfate; the mixture was allowed to stand in shade at 25℃ for 24 h before use. The prepared $ABTS^+$ solution (100 μL) was added to 96-well plate containing different concentrations of isolated compounds **1**–**3** and positive controls (at 20–200 μM), and mixed for 30 s. Plates were incubated for 30 min at 25℃. $ABTS^+$ radical scavenging capacity was subsequently read by ELISA reader (Infinite F200, Tecan Austria GmBH, Grodig, Austria) by examining the decrease in absorbance at 734 nm.[39]

The hydroxyl radical scavenging effect was estimated by applying the deoxyribose degradation assay, with slight modification.[37] Briefly, the antioxidant compounds **1**–**3** were first dissolved in EtOH to prepare sample dilutions (20–200 μM). Sample were added to a reaction solution containing 400 μL phosphate buffer (0.2 M, pH 7.4), 50 μL deoxyribose (50 mM), 50 μL EDTA (1 mM), 50 μL $FeCl_3$ (3.2 mM), 50 μL H_2O_2 (50 mM), and 50 μL ascorbic acid (1.8 mM). After incubation at 37℃ for 4 h, the reaction was terminated by adding 250 μL TCA. The color was then developed by adding 150 μL of TBA and heating in an oven at 100℃ for 15 min, subsequently cooled, and absorbance was recorded at 532 nm against the buffer.

The $ABTS^+$ and hydroxyl radical scavenging activities were calculated as follows: Radical scavenging activities (%) = $[1-(A_2/A_1)] \times 100$, where A_1 is test absorbance without sample and A_2 is test absorbance with compounds **1**−**3** or positive control ((+)-catechin).

Evaluation of pancreatic lipase inhibitory effect

The pancreatic lipase activity was evaluated using *p*-NPB as a substrate.[40] Briefly, an enzyme buffer was prepared by adding 5 μL (10 units) of pancreatic lipase from porcine, in enzyme buffer (1 mM EDTA, pH 6.8, 10 mM MOPS, pH 6.8) to 170 μL of Tris buffer (100 mM Tris-HCl, 5 mM $CaCl_2$, pH 7.0). Then, 20 μL of the compounds at the test concentration or positive control was mixed with 195 μL of the buffer and incubated for 20 min at 37℃. The amount of 5 μL of the substrate solution (15 mM of *p*-NPB in dimethylformamide) released by pancreatic lipase was measured with an ELISA reader at 405 nm. Inhibition of the pancreatic lipase activity was expressed as the percentage decrease in the absorbance when lipase was incubated with the test compounds. Orlistat was used as a positive control.

Supplementary Material

Supporting information for this article is available on the WWW under http://dx.doi.org/

Author Contribution Statement

G.H.J carried out experiment. G.H.J. and T.H.K. designed experiments, analyzed data and wrote the manuscript. Both authors read and approved the final manuscript.

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Chem. Biodiversity

TOC Graphic

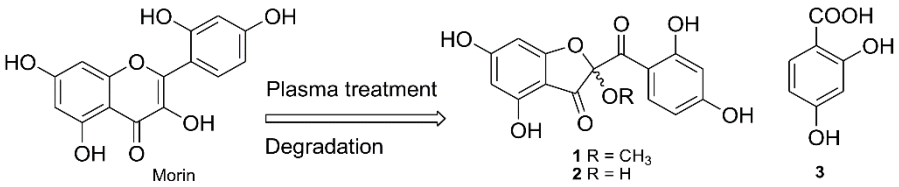

Table. Biological activities of newly generated compounds **1**-**3**

Compounds	IC_{50} value (μM)		
	ABTS^{+}	hydroxyl	Pancreatic lipase
Morin	>300	>300	>500
1	12.9±0.9	19.0±1.7	90.7±1.6
2	19.4±1.1	44.1±2.6	124.9±2.2
3	41.8±1.5	71.9±2.7	429.7±9.4