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Short communication

Synthesis and biological activity of novel tiliroside derivants

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ABSTRACT

A series of new tiliroside derivatives were synthesized and characterized by analytical ^{1}H NMR, ^{13}C NMR and mass spectrometry. All of the compounds were evaluated for anti-diabetic properties *in vitro* using HepG2 cells. Compounds **3c**, **3d**, and **3i**–**l** caused significant enhancements in glucose consumption by insulin-resistant HepG2 cells compared with control cells and cells that were exposed to metformin (an anti-diabetic drug). Moreover, compound **3l** significantly activated adenosine 5′-monophosphate-activated protein kinase activity and reduced acetyl-CoA carboxylase activity. Thus, the tiliroside derivative **3l** offers potential to be developed as a new approach for treating type II diabetes.

1. Introduction

Diabetes mellitus comprises a group of chronic metabolic disorders associated with the ability of the body to process carbohydrates, fats and proteins [1]. Currently, approximately 3% of the worldwide population is affected and global incidence is increasing [1]. Noninsulin-dependent diabetes mellitus (type II diabetes, T2D) is a heterogeneous disease characterized by hyperglycemia, which is caused by a disorder in insulin secretion, insulin resistance (IR) in target tissues, and activation of the hepatic glucose production pathway in the liver [2,3]. Amongst various pathological effects, the failure of hepatic control of glucose homeostasis is a key factor that causes hyperglycemia [4]. IR is characterized by the failure of tissues to respond to insulin, which results in reduced glucose intake in peripheral tissues and increased hepatic glucose output [5].

In recent years, plant extracts have been identified as a new avenue to explore for novel anti-diabetic drugs. Due to undesirable side effects associated with existing drugs, an increasing number of patients demand to use natural products with anti-diabetic activity [6]. Our previous study suggested that tiliroside, isolated from *Potentilla chinensis*, possessed significant anti-hyperglycemic effects compared with phenethyldiguanide in alloxan mice (Patent no.: CN ZL200610015591.5) [7]. The kaempferol-3-*O*-β-D-glucopyranose moiety of tiliroside shows only weak anti-diabetic activity [8], and so the cinnamoyl moiety of tiliroside is presumed to be the critical factor controlling its biological activity. In our previous work, the cinnamoyl moiety of tiliroside was modified [9]. However, a pharmacokinetics study showed that most of the tiliroside was quickly disintegrated into cinnamic acid and kaempferol-3-*O*-glucoside, which in turn was decomposed into kaempferol and glucose both in the blood and the intestines [10]. Based on these above observation, we designed a series of novel tiliroside derivatives, which could replace the glucose group and were connected to kaempferol by its CH_2 group (Fig. 1).

HepG2 cells are hepatocellular carcinoma cells that are valuable for investigating liver-associated functions. They maintain most functions of the liver and are stable during many passages [11,12]. IR in liver cells principally causes impaired glycogen synthesis and fails to suppress glucose production, which is the major contributing factor leading to hyperglycemia [13]. Numerous previous studies have used IR HepG2 cell to investigate T2D [14–17].

Adenosine 5′-monophosphate-activated protein kinase (AMPK) is considered to be a cellular energy sensor. It is important to understand the mechanism by which hepatic AMPK coordinates hepatic energy metabolism, as AMPK is a key master switch in

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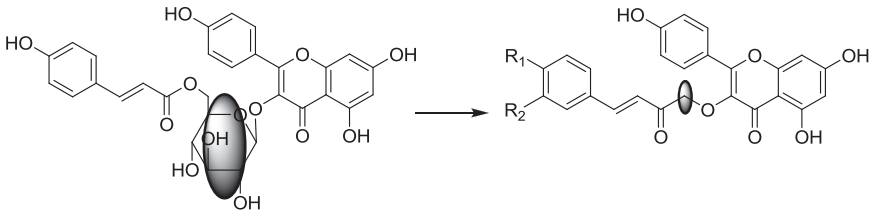


Fig. 1. The modifications of tiliroside.

regulating glucose and lipid metabolism in the liver [17,18]. Many flavonoids are reported to increase AMPK phosphorylation [19–21]. This present study concerns the synthesis of novel tiliroside derivatives, the study of their structure–activity relationships, and the potential anti-diabetic mechanism of these compounds on AMPK activity in human HepG2 hepatocytes.

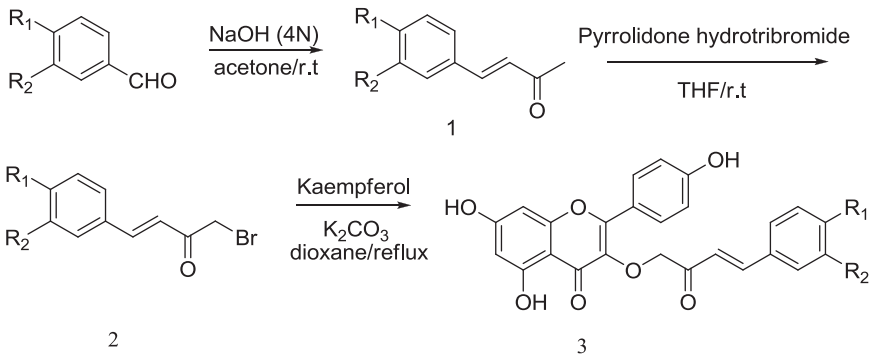
2. Chemistry

The target compounds were synthesized through three steps (Scheme 1). The first step was a Classin–Schimitt reaction from the substituted benzaldehyde to the α,β-unsaturated keto. Then the α-hydrogen atom in this keto was substituted by a bromine atom. Finally, the **3** compounds were prepared by the etherification reaction from the **2** compounds and kaempferol. The structures of compounds **3a**–**w** were determined by ^{1}H, ^{13}C NMR, and 2D NMR spectral data analyses, including COSY, HSQC, HMBC, and ROESY spectra.

3. Pharmacology

3.1. Glucose consumption assays in IR HepG2 cells

Human HepG2 cells were cultured in high-glucose DMEM supplemented with 10% fetal bovine serum. After achieving confluence, cells were cultured in 96-well cluster plates in the same medium for 24 h, before they were treated with 10^{-7} M insulin for 36 h in serum-free and phenol red-free high-glucose DMEM. After



	R_1	R_2		R_1	R_2
3a	OH	H	**3m**	CF_3	H
3b	OBn	H	**3n**	F	H
3c	OMe	H	**3o**	Cl	H
3d	OCH_2CH_3	H	**3p**	Br	H
3e	$O(CH_2)_3CH_3$	H	**3q**	SMe	H
3f	$O(CH_2)_5CH_3$	H	**3r**	OMe	OH
3g	$O(CH_2)_7CH_3$	H	**3s**	OH	OMe
3h	H	H	**3t**	OBn	OMe
3i	CH_3	H	**3u**	OH	Cl
3j	*i*-Pr	H	**3v**	Cl	Cl
3k	*t*-Bu	H	**3w**	OMe	Br
3l	CN	H			

Scheme 1. The synthesis of target compounds.

this incubation, the cells were washed four times with high-glucose DMEM (pH 4) and twice with phosphate-buffered saline. The cells were added to serum-free and phenol red-free high-glucose DMEM containing the various test compounds at different concentrations. The cells were incubated for 24 h, and then glucose content in the culture medium was measured using a glucose assay kit to assess the effects of the test compounds on glucose consumption by IR HepG2 cells. The enhancement ratio of glucose consumption (GC) was calculated as follows: GC % = (GC treatment group − GC of model group)/GC of model group × 100. The potencies of the products were expressed as median effective concentration (EC_{50}) values [13,22].

3.2. Western blotting

Human HepG2 cells were grown in 12-well plates. Cells were lysed on ice with 200 μl RIPA buffer (100 mM NaCl, 0.25% w/v sodium deoxycholate, 1.0% w/v NP40, 0.1% w/v sodium dodecyl sulfate (SDS), 2 mM ethylenediaminetetraacetic acid, 50 mM NaF, 10 nM okadaic acid, 1 mM sodium orthovanadate, protease inhibitor cocktail and 50 mM Tris–HCl, pH 7.2). Samples were electrophoresed on 7.5% SDS-polyacrylamide gels, and transferred to polyvinylidene fluoride membranes. The membranes were blocked for 1 h with 5% (w/v) bovine serum albumin, and incubated with the primary antibodies overnight at 4 °C, followed by incubation with appropriate secondary antibodies for 1 h at room temperature. Immunoblots were detected with chemiluminescent reagent and autoradiographic film.

4. Results

4.1. Glucose consumption in IR HepG2 cells

All test compounds were assayed for their effects on glucose consumption by IR HepG2 cells and potencies were compared using EC_{50} values. Compounds **3a**–**d**, **3h**–**l**, **3n** and **3p** significantly enhanced glucose consumption by IR HepG2 cells. Compounds **3c**, **3d**, and **3i**–**l** had greater anti-diabetic activity than the marketed anti-diabetic drug metformin (Table 1).

4.2. Western blotting assay

Western blotting analysis was use to evaluate the potential anti-diabetic mechanism of compound **3l**. Human HepG2 cells were treated with increasing doses of compound **3l** for 2 h. Compound **3l** stimulated the phosphorylation of AMPK and acetyl-CoA carboxylase (ACC) markedly in a dose-dependent fashion (Fig. 2). These results suggest that AMPK and ACC may be the key molecules in the signal transduction pathway that are targeted by compound **3l**.

Table 1
Effects on glucose consumption of compounds **3** in IR HepG2 cells.

Compound	EC_{50} (μM)	Compound	EC_{50} (μM)	Compound	EC_{50} (μM)
3a	0.368	**3j**	0.015	**3r**	5.070
3b	0.658	**3k**	0.013	**3s**	6.190
3c	0.149	**3l**	0.003	**3t**	>20
3d	0.042	**3m**	>20	**3u**	3.555
3e	>20	**3n**	0.669	**3v**	2.587
3f	>20	**3o**	1.075	**3w**	>20
3g	>20	**3p**	0.393	Metformin	0.270
3h	0.473	**3q**	>20	Tiliroside	0.155
3i	0.010				

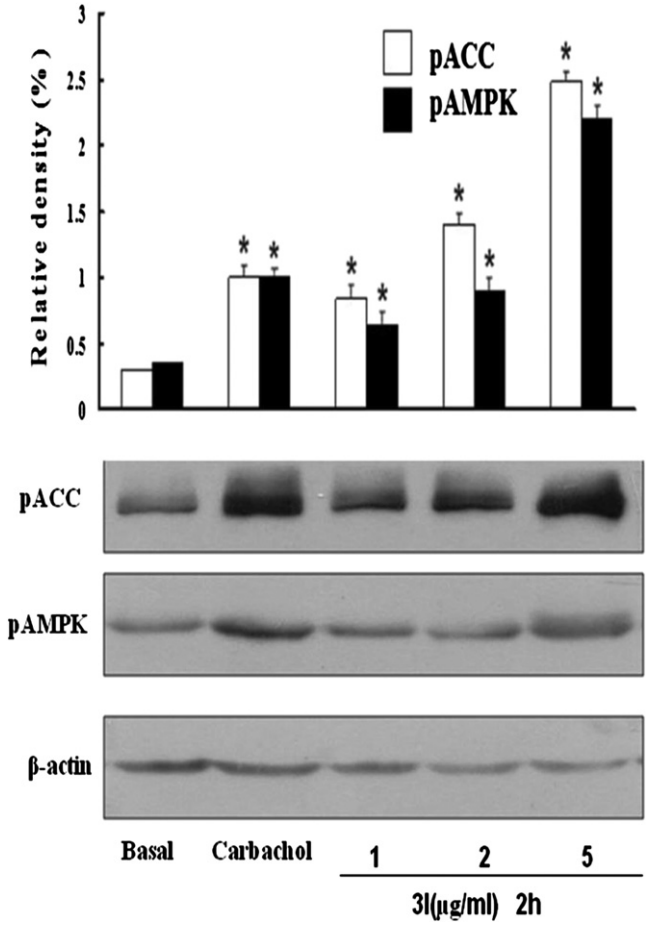


Fig. 2. Compound **3l** phosphorylates AMPK and ACC in human HepG2 cells. Results are the means ± SE of 3 independent experiments. $^{*}p < 0.05$ vs. basal.

5. Discussion

Most of the novel tiliroside derivants in this present study (the **3** series of compounds) at low concentrations improved glucose consumption by IR HepG2 cells. However, when the size of the substituted group (R_1) was significantly larger than OCH_2CH_3 or the R_2 was not an H atom, the activity of the compound was much lower, such as was the case for compounds **3e**, **3f**, **3g**, **3r**, **3s**, **3u** and **3v**. In addition, the activities of compounds with OCH_2CH_3, OCH_3 and CN substituted groups were greater than the OH group. Thus, we hypothesized that the amino residue of the binding site in the target protein may be a hydrophobic residue, and that the pocket is unable to hold groups larger than OCH_2CH_3. We also suggest that the unsaturated group (CN group) potentially provides a π–π interaction with the binding site, since compound **3l** displayed excellent activity.

Current research is focused on preventing or treating diabetes mellitus using substances present in natural products found in the diet or fruits. Flavonoids are ubiquitous components in vegetables and fruits, and are often consumed in large quantities during the daily diet. Many anti-diabetic flavonoids have been reported, including isoprenylated flavonoids from *Erythrina mildbraedii* that inhibit protein tyrosine phosphatase-1B with 50% inhibitory concentration (IC_{50}) values ranging from 14.8 to 39.7 μM [23]; 6-hydroxyluteolin, hypolaetin and quercetagetin inhibit dephosphorylated glycogen phosphorylase with IC_{50} values of 11.6, 15.7, and 9.7 μM, respectively [24]; and silibinin, which lowers glucose production from various gluconeogenic substrates in perifused rat hepatocytes at concentrations as low as 10 μM [25]. However, these

flavonoids show only weak activity compared with marketed anti-diabetic drugs. Our previous study demonstrated that tiliroside, isolated as a principal compound from *P. chinensis* extracts, displayed beneficial effects on hyperlipidemia due to diabetes, and showed significant anti-hyperglycemic, anti-hyperlipidemic, anti-oxidant activities in diabetic animals [26]. The novel tiliroside skeleton in compounds **3c**, **3d** and **3i**–**l** produced and described in this present study have significant anti-diabetic activities, with EC_{50} values ranging from 0.003 to 0.149 μM that are comparable in potency to metformin (Table 1).

AMPK is an energy sensor that regulates cellular metabolism that activates ACC [27]. In addition, AMPK is involved in the glucose metabolism of various tissues, including the liver, skeletal muscle, adipose tissues, and pancreatic β cells: which are key tissues in the pathogenesis of T2D [28]. The activation of AMPK in skeletal muscle, liver, and adipose tissues enhances metabolism, glucose uptake, insulin sensitivity, and oxidative metabolism of fatty acids, as well as increases in glucose transport and glycolysis. Thus, by inhibiting hepatic glucose output and increasing the liver stores of glucose due to causing its conversion to glycogen, AMPK could control systemic blood glucose levels in the body [29]. Therefore, a defect in AMPK signaling could account for many of the abnormalities observed in IR related to T2D [30].

As for the actions of flavonoid analogs in the AMPK signal transduction pathway, luteolin enhances the phosphorylation of AMPK and ACC at 10 and 20 μM in HepG2 cell lines, respectively [31]. The synthetic flavonoid S17834 persistently stimulates AMPK phosphorylation and activity at 10 μM in HepG2 cells [32], kaempferol and kaempferol-3-*O*-glucoside weakly enhance AMPK activity at 10 μM in 3T3-L1 cells [33], while naringenin increases muscle cell glucose uptake via AMPK at 75 μM [34]. In addition, 7-*O*-methylaromadendrin, isolated from *Inula viscose*, stimulates the reactivation of insulin-mediated phosphorylation of AMPK in HepG2 cells at 10 μM [35].

In a dose–response experiment (1 μg/ml [2.2 μM], 2 μg/ml and 5 μg/ml), compound **3l** not only activated AMPK but it also increased the phosphorylation of ACC (Fig. 2) at lower doses compared with each of the flavonoids mentioned above. Furthermore, in a previous report by our investigational team [36], we demonstrated that compound **3l** increased surface GLUT4myc levels and markedly stimulated the phosphorylation of AMPK and ACC in C2C12 and L6 muscle cells. Thus, compound **3l** with its novel skeleton could activate the AMPK signal pathway in three different cell lines, implicating a combination of downstream multi-target proteins in AMPK pathway.

The results in this present study suggest that tiliroside derivatives may be promising candidates in the development of new anti-diabetic lead compounds, and further biological evaluations are underway in our laboratory.

6. Conclusion

In summary, a novel series of tiliroside derivatives were synthesized. Among them compounds **3c**, **3d** and **3i**–**l** had significant anti-diabetic activities compared with the marketed anti-diabetic drug metformin [37]. Compound **3l** significantly activated AMPK activity, reduced ACC activity and enhanced glucose consumption in IR HepG2 cells.

7. Experimental protocols

7.1. Chemistry

All solvents and reagents were obtained from commercial sources and used without purification. Reactions were monitored by thin-layer chromatography (TLC) using pre-coated silica gel aluminum plates containing a fluorescence indicator. ^{1}H, ^{13}C NMR spectra were taken using a Bruker AV400 MHz. Chemical shifts of ^{1}H NMR spectra were recorded in parts per million with respect to tetramethylsilane (TMS), and the coupling constants (*J*) were measured in Hz. Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet). ^{1}H NMR spectra were recorded in $CDCl_3$ and DMSO-d_6 using TMS as internal standard. Mass spectra were taken in ESI mode on an Agilent 1200 LC-MS (Agilent, Palo Alto, CA, USA). The preparation of compounds **1a**–**w** and **2a**–**w** were in accordance with previous reports [38,39].

7.2. General procedure for the synthesis of compounds *3*

To a mixture of kaempferol (1.2 eq) and K_2CO_3 (1.2 eq) in 1,4-dioxane (10 ml), which was stirred for 90 min while maintaining gentle reflux, was added a solution of the **2** compounds (1 eq) in 1,4-dioxane (5 ml) for 30 min. The reaction mixture was refluxed until the starting material disappeared, as indicated by TLC (CH_2Cl_2:MeOH, 95:5). The solvent was removed from the reaction mixture under reduced pressure. Water was added, the aqueous phase was neutralized (to pH 7) with 1 M HCl, and then extracted with ethyl acetate. The organic phase was dried over anhydrous magnesium sulfate, filtered and concentrated. The crude product was purified using a gel-permeation chromatograph (HW-40; CH_2Cl_2:MeOH, 1:1) and prepared-TLC to give a yellow solid product.

7.2.1. 3-O-[(E)-4-(4-Hydroxyphenyl)-2-oxobut-3-en-1-yl] kaempferol (*3a*)

Yield 18.5%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.56 (1H, s, OH), 10.27 (1H, br s, OH), 10.12 (1H, br s, OH), 8.05 (2H, d, *J* = 8.9 Hz), 7.59 (1H, d, *J* = 16.1 Hz), 7.52 (2H, d, *J* = 8.6 Hz), 6.92 (2H, d, *J* = 8.9 Hz), 6.84 (1H, d, *J* = 16.1 Hz), 6.81 (2H, d, *J* = 8.6 Hz), 6.47 (1H, d, *J* = 2.1 Hz), 6.22 (1H, d, *J* = 2.0 Hz), 5.02 (2H, s). ^{13}C NMR (100 MHz, DMSO-d_6): δ 194.6, 178.0, 164.7, 161.6, 160.7, 156.8, 155.7, 143.6, 136.8, 131.2, 131.0, 125.6, 121.0, 119.3, 116.4, 116.0, 104.5, 99.1, 94.2, 75.6, 60.2. ESI-MS *m*/*z*: 445.3 $[M - H]^-$.

7.2.2. 3-O-[(E)-4-(4-(Benzyloxy)phenyl)-2-oxobut-3-en-1-yl] kaempferol (*3b*)

Yield 21.3%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.57 (1H, s, OH), 10.90 (1H, br s, OH), 10.28 (1H, br s, OH), 8.05 (2H, d, *J* = 8.9 Hz), 7.65–7.61 (3H, m), 7.47–7.39 (4H, m), 7.34 (1H, m), 7.07 (2H, d, *J* = 8.9 Hz), 6.94 (2H, m), 6.92 (1H, d, *J* = 16.2 Hz), 6.47 (1H, d, *J* = 2.0 Hz), 6.22 (1H, d, *J* = 2.0 Hz), 5.16 (2H, s), 5.04 (2H, s). ^{13}C NMR (100 MHz, DMSO-d_6): δ 194.7, 178.0, 164.7, 161.6, 161.0, 160.7, 156.8, 155.7, 143.0, 137.1, 136.8, 131.0, 130.9, 128.9, 128.4, 128.3, 127.4, 121.0, 120.5, 116.0, 115.8, 104.5, 99.1, 94.2, 75.6, 69.9. ESI-MS *m*/*z*: 535.5 $[M - H]^-$.

7.2.3. 3-O-[(E)-4-(4-Methoxyphenyl)-2-oxobut-3-en-1-yl] kaempferol (*3c*)

Yield 19.2%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.57 (1H, s, OH), 10.89 (1H, br s, OH), 10.29 (1H, br s, OH), 8.05 (2H, d, *J* = 8.9 Hz), 7.63 (1H, d, *J* = 16.4 Hz), 7.63 (2H, d, *J* = 8.8 Hz), 6.98 (2H, d, *J* = 8.8 Hz), 6.92 (2H, d, *J* = 8.9 Hz), 6.91 (1H, d, *J* = 16.4 Hz), 6.46 (1H, d, *J* = 2.0 Hz), 6.22 (1H, d, *J* = 2.0 Hz), 5.04 (2H, s), 3.80 (3H, s). ^{13}C NMR (100 MHz, DMSO-d_6): δ 194.7, 178.0, 164.7, 161.9, 161.6, 160.7, 156.8, 155.7, 143.1, 136.7, 131.0, 127.2, 121.0, 120.3, 116.0, 114.9, 104.5, 99.1, 94.2, 75.6, 55.8. ESI-MS *m*/*z*: 459.3 $[M - H]^-$.

7.2.4. 3-O-[(E)-4-(4-Ethoxyphenyl)-2-oxobut-3-en-1-yl] kaempferol (*3d*)

Yield 17.3%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.57 (1H, s, OH), 10.90 (1H, s, OH), 10.28 (1H, s, OH), 8.05 (2H, d, *J* = 8.7 Hz), 7.63 (1H,

d, J = 16.5 Hz), 7.61 (2H, d, J = 8.5 Hz), 7.29 (2H, d, J = 8.0 Hz), 6.97 (2H, d, J = 8.7 Hz), 6.93 (2H, d, J = 8.5 Hz), 6.90 (1H, d, J = 16.5 Hz), 6.47 (1H, d, J = 1.5 Hz), 6.22 (1H, d, J = 1.5 Hz), 5.04 (2H, s), 4.07 (2H, q, J = 6.9 Hz), 1.34 (3H, t, J = 6.9 Hz). ^{13}C NMR (100 MHz, DMSO-d_6): δ 194.2, 177.5, 164.2, 161.1, 160.7, 160.2, 156.3, 155.2, 142.6, 136.3, 130.5, 130.4, 126.5, 121.1, 120.5, 119.7, 115.5, 114.8, 104.0, 98.6, 93.7, 75.2, 63.3, 14.5. ESI-MS m/z: 473.4 [M − H]$^-$.

*7.2.5. 3-O-[(E)-4-(4-Butoxyphenyl)-2-oxobut-3-en-1-yl] kaempferol (**3e**)*

Yield 16.2%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.57 (1H, s, OH), 10.89 (1H, s, OH), 10.29 (1H, s, OH), 8.05 (2H, d, J = 8.8 Hz), 7.63 (1H, d, J = 16.9 Hz), 7.61 (2H, d, J = 8.2 Hz), 6.97 (2H, d, J = 8.8 Hz), 6.93 (2H, d, J = 8.2 Hz), 6.90 (1H, d, J = 16.9 Hz), 6.47 (1H, br s), 6.22 (1H, br s), 5.04 (2H, s), 4.07 (2H, t, J = 6.4 Hz), 1.73–1.66 (2H, m), 1.48–1.39 (2H, m), 0.93 (3H, t, J = 7.4 Hz). ^{13}C NMR (100 MHz, DMSO-d_6): δ 194.2, 177.5, 164.2, 161.1, 160.9, 160.2, 156.3, 155.2, 142.6, 136.3, 130.5, 130.4, 126.5, 120.5, 119.7, 115.5, 114.9, 104.0, 98.6, 93.7, 75.1, 67.4, 30.6, 18.7, 13.6. ESI-MS m/z: 501.5 [M − H]$^-$.

*7.2.6. 3-O-[(E)-4-(4-4-(Hexyloxy)phenyl)-2-oxobut-3-en-1-yl] kaempferol (**3f**)*

Yield 20.1%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.57 (1H, s, OH), 10.89 (1H, s, OH), 10.27 (1H, s, OH), 8.05 (2H, d, J = 8.4 Hz), 7.63 (1H, d, J = 16.9 Hz), 7.61 (2H, d, J = 8.4 Hz), 6.97 (2H, d, J = 8.4 Hz), 6.93 (2H, d, J = 8.4 Hz), 6.90 (1H, d, J = 16.9 Hz), 6.47 (1H, s), 6.22 (1H, s), 5.03 (2H, s), 4.00 (2H, t, J = 6.4 Hz), 1.73–1.68 (2H, m), 1.42–1.39 (2H, m), 1.31–1.30 (4H, m), 0.93 (3H, t, J = 6.7 Hz). ^{13}C NMR (100 MHz, DMSO-d_6): δ 194.2, 177.5, 164.2, 161.1, 160.9, 160.2, 156.3, 155.2, 142.6, 136.3, 130.5, 130.4, 126.5, 120.5, 119.7, 115.5, 114.9, 104.0, 98.6, 93.7, 75.1, 67.6, 30.9, 28.5, 25.1, 22.0, 13.6. ESI-MS m/z: 529.6 [M − H]$^-$.

*7.2.7. 3-O-[(E)-4-(4-4-(Octyloxy)phenyl)-2-oxobut-3-en-1-yl] kaempferol (**3g**)*

Yield 19.7%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.57 (1H, s, OH), 10.90 (1H, s, OH), 10.28 (1H, s, OH), 8.05 (2H, d, J = 8.8 Hz), 7.63 (1H, d, J = 16.9 Hz), 7.61 (2H, d, J = 8.2 Hz), 6.97 (2H, d, J = 8.8 Hz), 6.93 (2H, d, J = 8.2 Hz), 6.90 (1H, d, J = 16.9 Hz), 6.47 (1H, br s), 6.22 (1H, br s), 5.04 (2H, s), 4.00 (2H, t J = 7.0 Hz), 1.72–1.69 (2H, m), 1.39–1.38 (2H, m), 1.30–1.22 (8H, m), 0.93 (3H, t, J = 6.8 Hz). ^{13}C NMR (100 MHz, DMSO-d_6): δ 194.2, 177.5, 164.2, 161.1, 160.9, 160.2, 156.3, 155.2, 142.7, 136.3, 130.5, 130.4, 126.5, 120.5, 119.7, 115.5, 114.9, 104.0, 98.6, 93.7, 90.65, 75.1, 69.9, 67.6, 65.1, 62.7, 31.2, 28.7, 28.6, 28.5, 25.4, 22.1, 13.9. ESI-MS m/z: 557.6 [M − H]$^-$.

*7.2.8. 3-O-[(E)-(2-Oxo-4-phenylbut-3-en-1-yl)] kaempferol (**3h**)*

Yield 17.6%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.56 (1H, s, OH), 10.89 (1H, s, OH), 10.27 (1H, s, OH), 8.05 (2H, d, J = 8.8 Hz), 7.67 (1H, d, J = 16.4 Hz), 7.69–7.67 (2H, m), 7.45–7.43 (3H, m), 7.05 (1H, d, J = 16.4 Hz), 6.93 (2H, d, J = 8.8 Hz), 6.47 (1H, d, J = 1.9 Hz), 6.22 (1H, d, J = 1.9 Hz), 5.08 (2H, s). ^{13}C NMR (100 MHz, DMSO-d_6): δ 194.9, 178.0, 164.7, 161.6, 160.7, 156.8, 155.7, 143.1, 136.8, 134.7, 131.2, 131.0, 129.4, 129.0, 122.8, 121.0, 116.0, 104.5, 99.1, 94.2, 75.7. ESI-MS m/z: 429.4 [M − H]$^-$.

*7.2.9. 3-O-[(E)-(2-Oxo-4-(p-tolyl)but-3-en-1-yl)] kaempferol (**3i**)*

Yield 13.4%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.56 (1H, s, OH), 10.88 (1H, br s, OH), 10.27 (1H, br s, OH), 8.05 (2H, d, J = 8.9 Hz), 7.63 (1H, d, J = 16.3 Hz), 7.57 (1H, d, J = 8.0 Hz), 7.25 (2H, d, J = 8.0 Hz), 6.99 (1H, d, J = 16.3 Hz), 6.92 (2H, d, J = 8.9 Hz), 6.47 (1H, d, J = 2.0 Hz), 6.22 (1H, d, J = 2.0 Hz), 5.06 (2H, s), 2.34 (3H, s). ^{13}C NMR (100 MHz, DMSO-d_6): δ 194.9, 178.0, 164.7, 161.6, 160.7, 156.8, 155.7, 143.2, 141.3, 136.8, 132.0, 131.9, 131.0, 129.1, 121.8, 121.0, 116.0, 104.5, 99.1, 94.2, 75.7, 21.5. ESI-MS m/z: 443.4 [M − H]$^-$.

*7.2.10. 3-O-[(E)-4-(4-Isopropylphenyl)-2-oxobut-3-en-1-yl] kaempferol (**3j**)*

Yield 21.2%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.55 (1H, s, OH), 8.05 (1H, d, J = 8.9 Hz), 7.64 (1H, d, J = 16.3 Hz), 7.59 (2H, d, J = 8.2 Hz), 7.30 (2H, d, J = 8.2 Hz), 7.00 (1H, d, J = 16.3 Hz), 6.93 (2H, d, J = 8.9 Hz), 6.47 (1H, d, J = 2.0 Hz), 6.22 (1H, d, J = 2.0 Hz), 5.06 (2H, s), 2.91 (1H, sept, J = 6.9 Hz), 1.20 (6H, d, J = 6.9 Hz). ^{13}C NMR (100 MHz, DMSO-d_6): δ 194.9, 178.0, 164.7, 161.6, 160.7, 156.8, 155.7, 152.0, 143.2, 136.7, 132.3, 132.0, 131.0, 129.2, 127.4, 126.6, 116.0, 104.5, 99.1, 94.2, 75.7, 60.2, 33.9, 24.0. ESI-MS m/z: 471.2 [M − H]$^-$.

*7.2.11. 3-O-[(E)-4-(4-(tert-Butyl)phenyl)-2-oxobut-3-en-1-yl] kaempferol (**3k**)*

Yield 17.3%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.55 (1H, s, OH), 10.84 (1H, s, OH), 10.25 (1H, s, OH), 8.06 (2H, d, J = 8.8 Hz), 7.65 (1H, d, J = 16.3 Hz), 7.60 (2H, d, J = 8.4 Hz), 7.46 (2H, d, J = 8.4 Hz), 7.00 (1H, d, J = 16.3 Hz), 6.93 (2H, d, J = 8.8 Hz), 6.47 (1H, d, J = 2.2 Hz), 6.22 (1H, d, J = 2.0 Hz), 5.07 (2H, s), 1.29 (9H, s). ^{13}C NMR (100 MHz, DMSO-d_6) δ 194.9, 178.0, 164.7, 161.6, 160.7, 156.8, 155.7, 154.2, 143.1, 136.8, 132.0, 131.0, 128.9, 126.3, 122.0, 121.0, 116.0, 104.5, 99.1, 94.2, 75.7, 35.1, 31.3. ESI-MS m/z: 485.2 [M − H]$^-$.

*7.2.12. 3-O-[(E)-4-(4-Cyanophenyl)-2-oxobut-3-en-1-yl] kaempferol (**3l**)*

Yield 20.1%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.53 (1H, s, OH), 10.89 (1H, s, OH), 10.25 (1H, s, OH), 8.04 (2H, d, J = 8.9 Hz), 7.91–7.86 (4H, m), 7.70 (1H, d, J = 16.4 Hz), 7.19 (1H, d, J = 16.4 Hz), 6.91 (2H, J = 8.9 Hz), 6.47 (1H, d, J = 2.0 Hz), 6.22 (1H, d, J = 2.0 Hz), 5.10 (2H, s). ^{13}C NMR (100 MHz, DMSO-d_6) δ 195.0, 177.9, 167.6, 164.7, 163.5, 161.6, 160.7, 159.0, 156.8, 155.7, 142.4, 140.8, 139.3, 136.7, 133.2, 133.1, 131.0, 129.6, 126.0, 121.0, 119.0, 116.0, 112.9, 104.5, 99.2, 94.2, 75.9. ESI-MS m/z: 453.9 [M − H]$^-$.

*7.2.13. 3-O-[(E)-2-Oxo-4-(4-(trifluoromethyl)phenyl)but-3-en-1-yl] kaempferol (**3m**)*

Yield 22.4%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.53 (1H, s, OH), 10.89 (1H, s, OH), 10.25 (1H, s, OH), 8.04 (2H, d, J = 8.8 Hz), 7.90 (2H, d, J = 8.3 Hz), 7.79 (2H, d, J = 8.3 Hz), 7.74 (1H, d, J = 16.3 Hz), 7.18 (1H, d, J = 16.3 Hz), 6.92 (2H, d, J = 8.8 Hz), 6.48 (1H, d, J = 2.0 Hz), 6.22 (1H, d, J = 2.0 Hz), 5.10 (2H, s). ^{13}C NMR (100 MHz, DMSO-d_6): δ 195.0, 177.9, 167.4, 164.7, 161.6, 160.7, 156.8, 155.7, 141.1, 138.8, 136.7, 132.2, 132.0, 131.0, 129.6, 129.1, 126.3, 125.4, 121.0, 116.0, 104.5, 99.2, 94.2, 75.8, 71.6. ESI-MS m/z: 497.0 [M − H]$^-$.

*7.2.14. 3-O-[(E)-4-(4-Fluorophenyl)-2-oxobut-3-en-1-yl] kaempferol (**3n**)*

Yield 19.2%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.54 (1H, s, OH), 10.36 (1H, br s, OH), 10.00 (1H, br s, OH), 8.03 (2H, d, J = 8.4 Hz), 7.75–7.65 (3H, m), 7.29–7.25 (2H, m), 7.00 (1H, d, J = 16.0 Hz), 6.93 (2H, d, J = 8.4 Hz), 6.50 (1H, br s), 6.24 (1H, br s), 5.06 (2H, s). ^{13}C NMR (100 MHz, DMSO-d_6) δ 194.9, 177.9, 165.1, 164.9, 162.7, 161.6, 160.8, 156.8, 155.7, 141.9, 136.7, 131.4, 131.3, 131.0, 130.4, 122.8, 121.0, 116.6, 116.4, 116.0, 104.5, 99.2, 94.3, 75.7, 63.3. ESI-MS m/z: 447.1 [M − H]$^-$.

*7.2.15. 3-O-[(E)-4-(4-Chlorophenyl)-2-oxobut-3-en-1-yl] kaempferol (**3o**)*

Yield 12.4%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.60 (1H, s, OH), 10.97 (1H, br s, OH), 10.34 (1H, br s, OH), 8.10 (2H, d, J = 8.9 Hz), 7.76 (2H, d, J = 8.5 Hz), 7.71 (1H, d, J = 16.4 Hz), 7.55 (2H, d, J = 8.5 Hz), 7.12 (1H, d, J = 16.4 Hz), 6.97 (2H, d, J = 8.9 Hz), 6.53 (1H, d, J = 2.0 Hz), 6.27 (1H, d, J = 2.0 Hz), 5.13 (2H, s). ^{13}C NMR (100 MHz, DMSO-d_6): δ 194.9, 178.0, 164.7, 161.6, 160.7, 156.8, 155.7, 141.7, 136.7, 135.7, 133.7, 131.0, 130.7, 129.5, 123.5, 121.0, 116.0, 104.5, 99.2, 94.2, 75.8, 63.3. ESI-MS m/z: 463.8 [M − H]$^-$.

*7.2.16. 3-O-[(E)-4-(4-Bromophenyl)-2-oxobut-3-en-1-yl] kaempferol (**3p**)*

Yield 13.1%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.53 (1H, s, OH), 10.87 (1H, s, OH), 10.25 (1H, s, OH), 8.04 (2H, d, J = 8.7 Hz), 7.63 (4H, m), 7.07 (1H, d, J = 16.3 Hz), 6.92 (2H, d, J = 8.7 Hz), 6.47 (1H, d, J = 1.5 Hz), 6.22 (1H, d, J = 1.5 Hz), 5.07 (2H, s). ^{13}C NMR (100 MHz, DMSO-d_6): δ 194.9, 177.9, 164.7, 161.6, 160.7, 156.8, 155.7, 141.7, 136.7, 134.0, 132.4, 131.0, 130.9, 124.5, 123.6, 121.0, 116.0, 104.5, 99.2, 94.2. ESI-MS m/z: 507.6 [M − H]$^-$.

*7.2.17. 3-O-[(E)-4-(4-(Methylthio)phenyl)-2-oxobut-3-en-1-yl] kaempferol (**3q**)*

Yield 16.5%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.56 (1H, s, OH), 10.90 (1H, s, OH), 10.28 (1H, s, OH), 8.05 (2H, d, J = 8.8 Hz), 7.63 (1H, d, J = 16.0 Hz), 7.61 (2H, d, J = 8.0 Hz), 7.29 (2H, d, J = 8.0 Hz), 7.00 (1H, d, J = 16.0 Hz), 6.93 (2H, d, J = 8.8 Hz), 6.47 (1H, br s), 6.22 (1H, br s), 5.06 (2H, s), 2.51 (3H, s). ^{13}C NMR (100 MHz, DMSO-d_6): δ 194.3, 177.5, 164.2, 161.1, 160.2, 156.3, 155.2, 142.2, 136.3, 130.5, 130.4, 129.0, 125.5, 121.1, 120.5, 115.5, 104.0, 98.6, 93.7, 75.2, 14.1. ESI-MS m/z: 475.5 [M − H]$^-$.

*7.2.18. 3-O-[(E)-4-(3-Hydroxy-4-methoxyphenyl)-2-oxobut-3-en-1-yl] kaempferol (**3r**)*

Yield 12.4%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.57 (1H, s, OH), 10.89 (1H, s, OH), 10.27 (1H, s, OH), 9.25 (1H, s, OH), 8.05 (2H, d, J = 8.4 Hz), 7.55 (1H, d, J = 16.0 Hz), 7.12 (2H, m), 6.97 (1H, d, J = 8.4 Hz), 6.92 (2H, d, J = 8.4 Hz), 6.82 (1H, d, J = 16.0 Hz), 6.47 (1H, br s), 6.22 (1H, br s), 5.02 (2H, s), 3.82 (3H, s). ^{13}C NMR (100 MHz, DMSO-d_6): δ 194.1, 177.5, 164.2, 161.1, 160.2, 156.3, 155.2, 150.4, 146.7, 143.1, 136.3, 130.5, 127.0, 121.8, 120.5, 119.7, 115.5, 114.2, 112.0, 104.0, 98.6, 93.7, 75.1, 55.6. ESI-MS m/z: 475.4 [M − H]$^-$.

*7.2.19. 3-O-[(E)-4-(3-Methoxy-4-hydroxyphenyl)-2-oxobut-3-en-1-yl] kaempferol (**3s**)*

Yield 13.5%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.58 (1H, s, OH), 10.89 (1H, s, OH), 10.26 (1H, s, OH), 9.70 (1H, br s, OH), 8.06 (2H, d, J = 8.8 Hz), 7.59 (1H, d, J = 16.1 Hz), 7.30 (1H, d, J = 1.5 Hz), 7.13 (1H, dd, J = 8.2, 1.5 Hz), 6.92 (2H, d, J = 8.8 Hz), 6.90 (1H, d, J = 16.1 Hz), 6.47 (1H, d, J = 2.0 Hz), 6.22 (1H, d, J = 2.0 Hz), 5.04 (2H, s), 3.82 (3H, s). ^{13}C NMR (100 MHz, DMSO-d_6): δ 194.5, 178.0, 164.7, 161.6, 160.7, 156.8, 155.7, 150.2, 148.4, 144.0, 136.8, 131.0, 126.2, 124.0, 121.0, 119.7, 116.1, 116.0, 112.0, 104.5, 99.1, 94.2, 75.5, 56.1. ESI-MS m/z: 475.4 [M − H]$^-$.

*7.2.20. 3-O-[(E)-4-(3-Methoxy-4-benzyloxy phenyl)-2-oxobut-3-en-1-yl] kaempferol (**3t**)*

Yield 22.5%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.58 (1H, s, OH), 10.90 (1H, s, OH), 10.27 (1H, s, OH), 8.06 (2H, d, J = 8.0 Hz), 7.62 (1H, d, J = 16.2 Hz), 7.46–7.7.35 (6H, m), 7.23 (1H, d, J = 8.4 Hz), 7.09 (1H, d, J = 8.4 Hz), 6.97 (1H, d, J = 16.2 Hz), 6.92 (2H, d, J = 8.0 Hz), 6.47 (1H, br s), 6.22 (1H, br s), 5.14 (2H, s), 5.07 (2H, s), 3.82 (3H, s). ^{13}C NMR (100 MHz, DMSO-d_6): δ 194.1, 177.5, 164.2, 161.1, 160.2, 156.3, 155.2, 150.2, 149.2, 143.0, 136.6, 136.3, 130.5, 128.4, 127.9, 127.2, 123.2, 120.5, 115.5, 113.1, 110.8, 104.0, 98.6, 93.7, 75.0, 69.8, 55.6. ESI-MS m/z: 565.5 [M − H]$^-$.

*7.2.21. 3-O-[(E)-4-(3-Hydroxy-4-chlorophenyl)-2-oxobut-3-en-1-yl] kaempferol (**3u**)*

Yield 13.5%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.57 (1H, s, OH), 10.89 (2H, s, OH), 10.28 (1H, s, OH), 8.06 (2H, d, J = 8.6 Hz), 7.75 (1H, s), 7.58 (1H, d, J = 16.2 Hz), 7.50 (1H, d, J = 8.5 Hz), 7.02 (1H, d, J = 8.5 Hz), 6.93 (2H, d, J = 8.6 Hz), 6.92 (1H, d, J = 16.2 Hz), 6.48 (1H, br s), 6.22 (1H, br s), 5.04 (2H, s). ^{13}C NMR (100 MHz, DMSO-d_6): δ 194.1, 177.5, 164.2, 161.1, 160.2, 156.3, 155.5, 155.2, 141.6, 136.2, 130.5, 130.3, 128.8, 126.5, 120.5, 120.4, 120.3, 116.9, 115.5, 104.0, 98.6, 93.7, 75.1. ESI-MS m/z: 479.9 [M − H]$^-$.

*7.2.22. 3-O-[(E)-4-(3,4-Dichlorophenyl)-2-oxobut-3-en-1-yl] kaempferol (**3v**)*

Yield 24.5%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.54 (1H, s, OH), 10.90 (1H, s, OH), 10.26 (1H, s, OH), 8.05 (2H, d, J = 8.6 Hz), 8.02 (1H, br s), 7.69 (2H, m), 7.63 (1H, d, J = 16.3 Hz), 7.15 (1H, d, J = 16.3 Hz), 6.91 (2H, d, J = 8.6 Hz), 6.47 (1H, br s), 6.22 (1H, br s), 5.08 (2H, s). ^{13}C NMR (100 MHz, DMSO-d_6): δ 194.4, 177.4, 170.3, 164.2, 161.1, 160.2, 156.3, 155.2, 139.7, 136.2, 135.1, 132.8, 131.8, 131.0, 130.5, 130.2, 128.3, 124.3, 120.5, 115.5, 112.8, 104.0, 98.6, 93.7, 75.3, 59.7. ESI-MS m/z: 498.3 [M − H]$^-$.

*7.2.23. 3-O-[(E)-4-(3-Bromo-4-methoxyphenyl)-2-oxobut-3-en-1-yl] kaempferol (**3w**)*

Yield 19.5%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.61 (1H, s, OH), 10.93 (1H, br s, OH), 10.32 (1H, br s, OH), 8.10 (2H, d, J = 8.8 Hz), 8.02 (1H, s), 7.74 (1H, d, J = 8.8 Hz), 7.64 (1H, d, J = 16.4 Hz), 7.21 (1H, d, J = 8.8 Hz), 7.04 (1H, d, J = 16.4 Hz), 6.96 (2H, d, J = 8.8 Hz), 6.52 (1H, br s), 6.26 (1H, br s), 5.10 (2H, s), 3.95 (3H, s). ^{13}C NMR (100 MHz, DMSO-d_6): δ 194.1, 177.5, 164.2, 161.1, 160.2, 157.1, 156.3, 155.1, 141.0, 136.2, 132.8, 130.5, 129.9, 128.6, 128.3, 121.3, 120.5, 115.5, 112.8, 111.2, 104.0, 98.6, 93.7, 75.2, 56.5. ESI-MS m/z: 538.2 [M − H]$^-$.

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