SUPPORTING INFORMATION
IDENTIFICATION, SYNTHESIS AND PHOTO-PROTECTION EVALUATION OF ARYLTHIAZOLE DERIVATIVES AS A NOVEL SERIES OF SUNSCREENS

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Contents

2-(2-Ethyl-4-pyridinyl)-4-methyl-*N*-phenyl-5-thiazolecarboxamide (4a). To a solution of compound **3b** (124 mg, 0.50 mmol) in 5 mL of dry DMF was added EDC·HCl (125 mg, 0.65 mmol) and HOBt (74 mg, 0.55 mol) at 0 ^{o}C, and the mixture was stirred for 10 min, and then aniline (50 μL, 0.55 mmol) was added and the reaction mixture was warm to room temperature for stirring 3 h. The reaction was quenched with ice water and then extracted with EtOAc. The organic layer was

washed with water and brine twice and then dried over anhydrous Na_2SO_4. The product was purified by silica gel column chromatography (petroleum ether/EtOAc = 1/1) to give compound **4a** (137 mg, 85% yield) ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.61 (d, *J* = 5.1 Hz, 1H), 7.80 (s, 1H), 7.68 (s, 1H), 7.60 (d, *J* = 5.1 Hz, 1H), 7.57 (d, *J* = 7.8 Hz, 2H), 7.37 (dd, *J* = 7.8, 7.5 Hz, 2H), 7.17 (dd, *J* = 7.5, 7.5 Hz, 1H), 2.86 (q, *J* = 7.5 Hz, 2H), 2.80 (s, 3H), 1.35 (t, *J* = 7.5 Hz, 3H).

***N*-(4-Bromophenyl)-2-(2-ethyl-4-pyridinyl)-4-methyl-5-thiazolecarboxamide (4b).** The title compound was prepared according to **4a** except using 4-bromoaniline instead of aniline. Yield 85%: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.64 (d, *J* = 5.1 Hz, 1H), 7.70 (s, 1H), 7.59 (d, *J* = 5.1 Hz, 1H), 7.54 (s, 1H), 7.50–7.49 (m, 4H), 2.91 (q, *J* = 7.8 Hz, 2H), 2.82 (s, 3H), 1.37 (t, *J* = 7.8 Hz, 3H).

2-(2-Ethyl-4-pyridinyl)-*N*-(3-methoxyphenyl)-4-methyl-5-thiazolecarboxamide (4c). The title compound was prepared according to **4a** except using 3-methoxyaniline instead of aniline. Yield 92%: ^{1}H NMR (300 MHz, DMSO): δ 10.33 (s, 1H), 8.64 (d, *J* = 5.1 Hz, 1H), 7.79 (s, 1H), 7.72 (d, *J* = 5.1Hz, 1H), 7.36 (s, 1H), 7.27 (d, *J* = 5.1 Hz, 2H), 6.27 (dd, *J* = 5.1, 5.1 Hz, 1H), 3.76 (s, 3H), 2.86 (q, *J* = 7.5 Hz, 2H), 2.67 (s, 3H), 1.28 (t, *J* = 7.5 Hz, 3H).

2-(2-ethyl-4-pyridinyl)-*N*-(2-methoxyphenyl)-4-methyl-5-thiazolecarboxamide (4d). The title compound was prepared according to **4a** except using 2-methoxyaniline instead of aniline. Yield 70%: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.64 (d, *J* = 5.1 Hz , 1H), 8.43 (d, *J* = 7.8 Hz, 1H), 8.34 (s, 1H), 7.71 (s, 1H), 7.62 (d, *J* = 5.1 Hz, 1H), 7.12 (dd, *J* = 7.8, 7.8 Hz, 1H), 7.03 (dd, *J* = 7.8, 7.8Hz, 1H), 6.94(d, *J* = 7.8 Hz, 1H), 3.95 (s, 3H), 2.92 (q, *J* = 7.5 Hz, 2H), 2.85 (s, 3H), 1.37 (t, *J* = 7.5 Hz, 3H).

***N*-(Methoxycarbonylethyl)-2-(2-ethyl-4-pyridinyl)-4-methyl-5-thiazolecarboxamide (5b).** The title compound was prepared according to **5a** except using methyl 3-aminopropanoate hydrochloride instead of glycine ethyl ester hydrochloride. Yield 95%: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.62 (d, *J* = 5.1 Hz, 1H), 7.67 (s, 1H), 7.57 (d, *J* = 5.1 Hz, 1H), 6.71 (br s, 1H), 3.73–3.68 (m, 5H), 2.90 (q, *J* = 7.2 Hz, 2H), 2.75 (s, 3H), 2.70 (t, *J* = 5.7 Hz, 2H), 1.35 (t, *J* = 7.2 Hz, 3H).

***N*-(Ethoxycarbonylpropyl)-2-(2-ethyl-4-pyridinyl)-4-methyl-5-thiazolecarboxamide (5c).** The title compound was prepared according to **5a** except using ethyl 4-aminobutanoate instead of glycine ethyl ester hydrochloride. Yield 53%: ^{1}H NMR (300 MHz,$CDCl_3$): δ 8.59 (d, *J* = 5.4 Hz, 1H), 7.71 (s, 1H),7.60 (d, *J* = 5.4 Hz, 1H), 6.45 (br s, 1H), 4.14 (q, *J* = 7.2 Hz, 2H), 3.52–3.44 (m, 2H), 2.89 (q, *J* = 7.2 Hz, 2H), 2.75 (s, 3H), 2.45 (t, *J* = 6.9 Hz, 2H), 1.96 (m, 2H), 1.34 (t, *J* = 7.2 Hz, 3H), 1.25 (t, *J* = 7.2 Hz, 3H).

***N*-(Ethoxycarbonylpentyl)-2-(2-ethyl-4-pyridinyl)-4-methyl-5-thiazolecarboxamide (5d).** The title compound was prepared according to **5a** except using ethyl 6-aminohexanoate instead of glycine ethyl ester hydrochloride. Yield 61%: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.61 (d, *J* = 5.1 Hz, 1H), 7.67 (s, 1H), 7.55 (d, *J* = 5.1 Hz, 1H), 5.94 (br s, 1H),4.12 (q, *J* = 6.9 Hz, 2H), 3.49–3.41 (m, 1H), 2.90 (q, *J* = 7.5 Hz, 2H), 2.75 (s, 3H), 2.33 (t, *J* = 6.9 Hz, 2H), 1.72–1.60 (m, 6H), 1.35 (t, *J* =7.5 Hz, 3H), 1.25 (t, *J* = 6.9 Hz, 3H).

***N*-(Ethoxycarbonylmethyl)-2-(4-pyridinyl)-4-methyl-5-thiazolecarboxamide (6a).** The title compound was prepared according to **5a** except using compound **3a** instead of **3b**. Yield 64%: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.73 (d, *J* = 5.1 Hz, 2H), 7.79 (d, *J* = 5.1Hz, 2H), 6.43 (br s, 1H), 4.28 (q, *J* = 7.2 Hz, 2H), 4.23 (d, *J* = 4.8 Hz, 2H), 2.79 (s, 3H), 1.33 (t, *J* = 7.2 Hz, 3H).

***N*-(Ethoxycarbonylmethyl)-2-(2-propyl-4-pyridinyl)-4-methyl-5-thiazolecarboxamide (6b).** The title compound was prepared according to **5a** except using compound **3c** instead of **3b**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.62 (d , *J* = 5.1 Hz, 1H) , 7.66 (s, 1H) , 7.57 (d , *J* = 5.1 Hz , 1H), 6.46 (br s, 1H), 4.27 (q , *J* = 7.2 Hz, 2H), 4.22 (d , *J* = 4.8 Hz, 2H), 2.82 (t, *J* = 7.5 Hz, 2H), 2.78 (s, 3H), 1.80 (m, 2H), 1.32 (t, *J* = 7.2Hz, 3H), 0.98 (t, *J* = 7.2 Hz, 3H).

***N*-(Ethoxycarbonylmethyl)-2-(2-butyl-4-pyridinyl)-4-methyl-5-thiazolecarboxamide (6c).** The title compound was prepared according to **5a** except using compound **3d** instead of **3b**. Yield 46%: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.62 (d, *J* = 5.1 Hz, 1H), 7.67 (s, 1H), 7.57 (d, *J* = 5.1 Hz, 1H), 6.44 (br s, 1H), 4.46 (q, *J* = 6.9 Hz, 2H), 4.22 (d, 2H), 2.86 (t, *J* = 8.1 Hz, 2H), 2.79 (s, 3H), 1.45–1.32 (m, 7H), 0.95 (t, *J* = 7.2 Hz,

3H).

2-((2-(2-Ethyl-4-pyridinyl)-4-methyl-5-thiazolyl)methoxy)-*N*-phenylacetamide (9a). The title compound was prepared according to **4a** except using 2-((2-(2-ethyl-4-pyridinyl) -4-methyl-5-thiazolyl) methoxy) acetic acid instead of **3b**. Yield 44%.: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.60 (d, *J* = 5.1 Hz, 1H), 8.20 (s, 1H), 7.67 (s, 1H), 7.57–7.54 (m, 3H), 7.36–7.26 (m, 2H), 7.16–7.11 (m, 1H), 4.84 (s, 2H), 4.15 (S, 2H), 2.92(q, *J* = 7.5 Hz, 2H), 2.52 (s, 3H), 1.35 (t, *J* = 7.5 Hz, 3H).

***N*-Benzyl-2-((2-(2-ethyl-4-pyridinyl)-4-methyl-5-thiazolyl)methoxy)acetamide (9b).** The title compound was prepared according to **9a** except using benzylamine instead of aniline. Yield 42%: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.59 (d, *J* = 5.1 Hz, 1H), 7.64 (s, 1H), 7.52 (d, *J* = 5.1 Hz, 1H), 6.79 (br s, 1H), 4.74 (S, 2H), 4.50 (d, *J* = 5.0 Hz 2H), 4.09 (s, 2H), 2.89 (q, *J* = 7.2 Hz, 2H), 2.46 (s, 3H), 1.36 (t, *J* = 7.2 Hz, 3H).

2-((2-(2-Ethyl-4-pyridinyl)-4-methyl-5-thiazolyl)methoxy)-*N*-phenethylacetamide (9c). The title compound was prepared according to **9a** except using phenylethylamine instead of aniline. Yield 35%: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.61 (d, *J* = 5.1 Hz, 1H), 7.32 (s, 1H), 7.32 (d, *J* = 5.1 Hz, 1H), 6.53 (br s, 1H), 4.67 (s, 2H), 4.01 (s, 2H), 3.58 (q, *J* = 6.3 Hz, 2H), 2.92–2.82 (m, 4H), 2.44 (s, 3H), 1.37 (t, *J* = 6.3 Hz, 3H).

***N*-(Ethoxycarbonylmethyl)-2-(4-trifluoromethylphenyl)-4-methyl-5-thiazolecarboxamide (11b).** The title compound was prepared according to **5a** except using compound **10b** instead of **3b**. Yield 80%: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.06 (d, *J* = 7.8 Hz, 2H), 7.71 (d, *J* = 7.8 Hz, 2H), 6.42–6.37 (m, 1H), 4.30 (q, *J* = 7.2 Hz, 2H), 4.23 (d, *J* = 5.1 Hz, 2H), 2.79 (s, 3H), 1.33 (t, *J* = 7.2 Hz, 3H).

***N*-(Ethoxycarbonylmethyl)-2-(4-methoxyphenyl)-4-methyl-5-thiazolecarboxamide (11c).** The title compound was prepared according to **5a** except using compound **10c** instead of **3b**. Yield 82%: ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.88 (d, *J* = 8.7 Hz, 2H), 6.95 (d, *J* = 8.7 Hz, 2H), 6.32 (br s, 1H), 4.27 (q, *J* = 7.2 Hz, 2H) , 4.20 (d, *J* = 4.8 Hz, 2H), 3.86 (s, 3H), 2.75 (s, 3H), 1.32 (t, *J* = 7.2 Hz, 3H).

***N*-(Ethoxycarbonylmethyl)-2-(4-chlorophenyl)-4-methyl-5-thiazolecarboxamide (11d).** The title compound was prepared according to **5a** except using compound **10d**

instead of **3b**. Yield 83%: ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.88 (d, *J* = 8.7 Hz, 2H), 7.43 (d, 2H), 6.37 (br s, 1H), 4.28 (q, *J* = 7.2 Hz, 2H), 4.22 (d, *J* = 4.8 Hz, 2H), 2.76 (s, 3H), 1.33 (t, *J* = 7.2 Hz, 3H).

***N*-(Ethoxycarbonylmethyl)-2-(2-chlorophenyl)-4-methyl-5-thiazolecarboxamide (11e).** The title compound was prepared according to **5a** except using compound **10e** instead of **3b**. Yield 79%: ^{1}H NMR (300 MHz, $CDCl_3$): 8.29–8.27 (m, 1H), 7.52–7.49 (m, 1H), 7.40–7.37 (m, 2H), 6.41 (br s, 1H), 4.28 (q, *J* = 7.2 Hz, 2H), 4.23 (d, *J* = 4.8 Hz, 2H), 2.80 (s, 3H), 1.33 (t, *J* = 7.2 Hz, 3H).

***N*-(Ethoxycarbonylmethyl)-2-(2,6-dichlorophenyl)-4-methyl-5-thiazolecarboxamide (11f).** The title compound was prepared according to **5a** except using compound **10f** instead of **3b**. Yield 45%: ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.44–7.41 (m, 2H), 7.37–7.33 (m, 1H), 6.42 (s, 1H), 4.28 (q, *J* = 5.4 Hz, 2H), 4.23 (d, *J* = 3.9 Hz, 2H), 2.82 (s, 3H), 1.33 (t, *J* = 5.4 Hz, 2H).

***N*-(Ethoxycarbonylmethyl)-5-phenyl-2-furancarboxamide (13a).** The title compound was prepared according to **5a** except using compound **12a** instead of **3b**. Yield 49%: ^{1}H NMR (300 MHz, DMSO): δ 8.64 (d, *J* = 5.1 Hz, 1H), 8.23 (d, 1H), 7.70 (br s, 1H), 7.64 (s, 2H), 4.31–4.24 (m, 4H), 2.92 (q, *J* = 7.5 Hz, 2H), 1.36–1.33 (m, 6H).

N-(Ethoxycarbonylmethyl)-5-phenyl-2-thiophenecarboxamide (13b). The title compound was prepared according to **5a** except using compound **12b** instead of **3b**. Yield 64%: ^{1}H NMR (300 MHz, $CDCCl_3$): δ 7.57 (d, *J* = 1.5 Hz, 1H), 7.46 (d, *J* = 1.5 Hz, 1H), 7.37–7.27(m, 3H), 7.21–7.19 (m, 2H), 6.43 (br s, 2H), 4.20 (q, *J* = 7.2 Hz, 2H), 4.16 (d, *J* = 5.1 Hz, 2H), 1.25 (t, *J* = 7.2 Hz, 3H).

Cell culture

The human keratinocyte cell line HaCaT was cultured in RPMI-1640 supplemented with 10% heat-inactivated fetal calf serum, 50 IU/ml penicillin, 50 mg/ml streptomycin and 2 mM glutamine at 37 ^{o}C in a humidified 5% CO_2 incubator.

Cell treatments and MTS assay

To assess the photo-protective effects of the compounds, 5000/well HaCaT cells cultured in 96-well were pretreated with compounds that were dissolved in DMSO as stock solution at the indicated concentrations for 6 hours, respectively. The control group was carried out with DMSO applied without compounds and polydatin and BP-3 were used as positive controls. Cells were then irradiated with UVB (dose, 30mJ/cm^2) for about 1 min by Bio-Sun system (Vilber Lourmat, Marne-la-Vallée, France) at wavelength of 312 nm and incubated in the serum-free culture medium. After 24 hours, cell viability was assessed using the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium bromide (MTS) (Promega Corporation, Madison, WI) assay[1].

For the cytotoxicity experiment, 5000/well HaCaT cells were cultured in 96-well plates and incubated with indicated compounds for 48 hours. Cell viability was also assessed using the MTS.

UVB irradiation in animal model

For *in vivo* studies, the ointment containing 1.0 mM compound **5a** was applied on the right side of BALB/c-nu mice dorsal skin once every 12 hours while the left side as a control was treated with ointment without drug. One hour later, the animals were irradiated with Bio-Sun system at wavelength 312nm for UVB (dose, 360mJ/cm^2) once a day for 7 days.[2]

Histology

Mice skin specimens were excised after treatment. Samples were immediately fixed in 10% neutral buffered formaldehyde for 24 hours, progressively dehydrated in solutions containing an increasing percentage of ethanol (75, 85, 95 and 100%, v/v), cleared in Histoclear (AS-ONE, Tokyo, Japan), and stained with hematoxylin-eosin (HE) to indicate nucleus and cytoplasm, respectively.

DPPH radical assay

The free radical scavenging capacity of compound 5a was assessed in 96-well plates

using the DPPH radical assay.[3] The reaction mixture contained 100μM DPPH and various concentrations of the test substances (dissolved and diluted in ethyl alcohol). After 30 min incubation in the dark at 37℃, absorption was recorded at 517 nm in a microplate reader. The radical scavenging activity was determined using the following equation: Radical scavenging activity (%) = [(1−ODsample)/ ODcontrol] × 100.

Statistical analysis

All the data in the experiments are more than three replications and all values are expressed as means ± standard error (SE). The results were subjected to an analysis of variance using student's t-test to assess the statistical significance of differences. $P < 0.05$ was considered statistically significant.

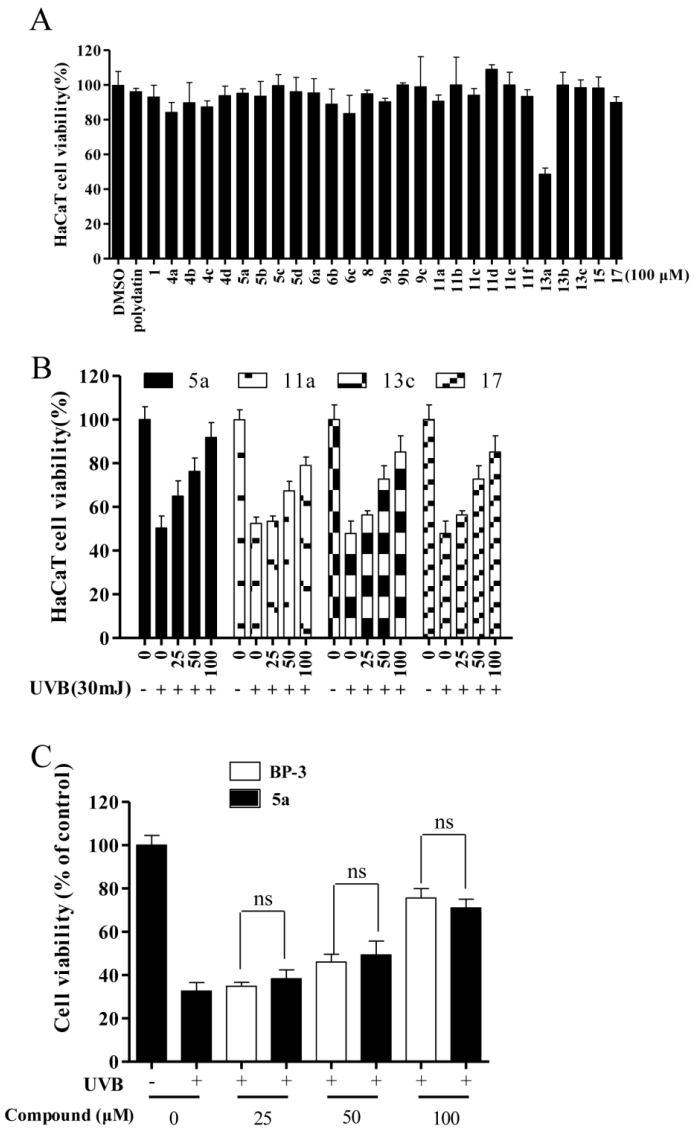

Figure S1. (A) The cytotoxicity of arylthiazole derivatives on human keratinocyte cells (HaCaT). (B) Protective effect of compound **5a**, **11a**, **13c**, **17** on HaCaT cells from UV-B-induced cell death. (C) Compared compound 5a with BP-3 (a positive control) for effect of protecting HaCaT cells from UVB-induced cell death. (n = 5, ns = no significant difference).

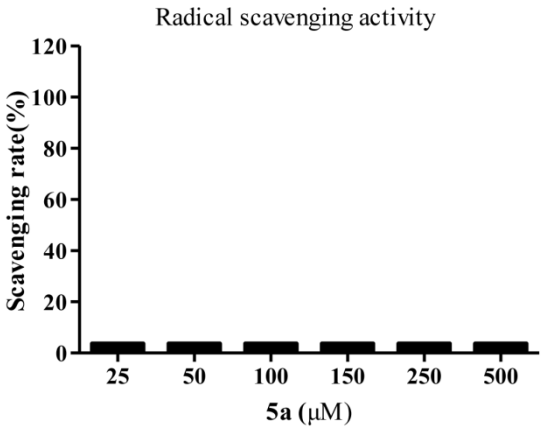

Figure S2. Radical scavenging activity of compound **5a** measured using DPPH radical assay (n = 5).

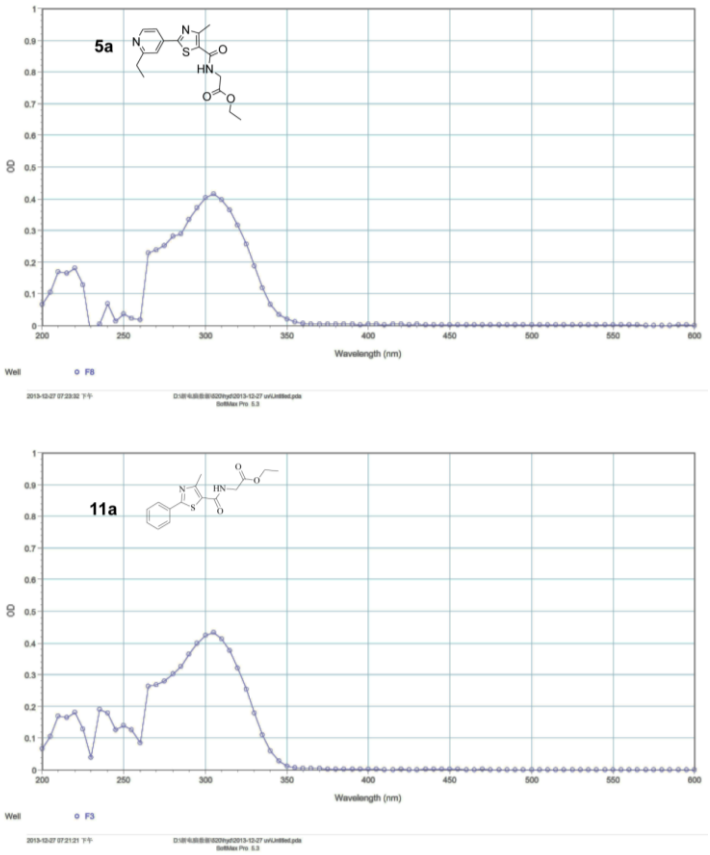

Figure S3A. Ultraviolet absorption spectrum of compound **5a** and **11a** (100 μM) under irradiation (Spectra MAX 190, Molecular Devices, CA).

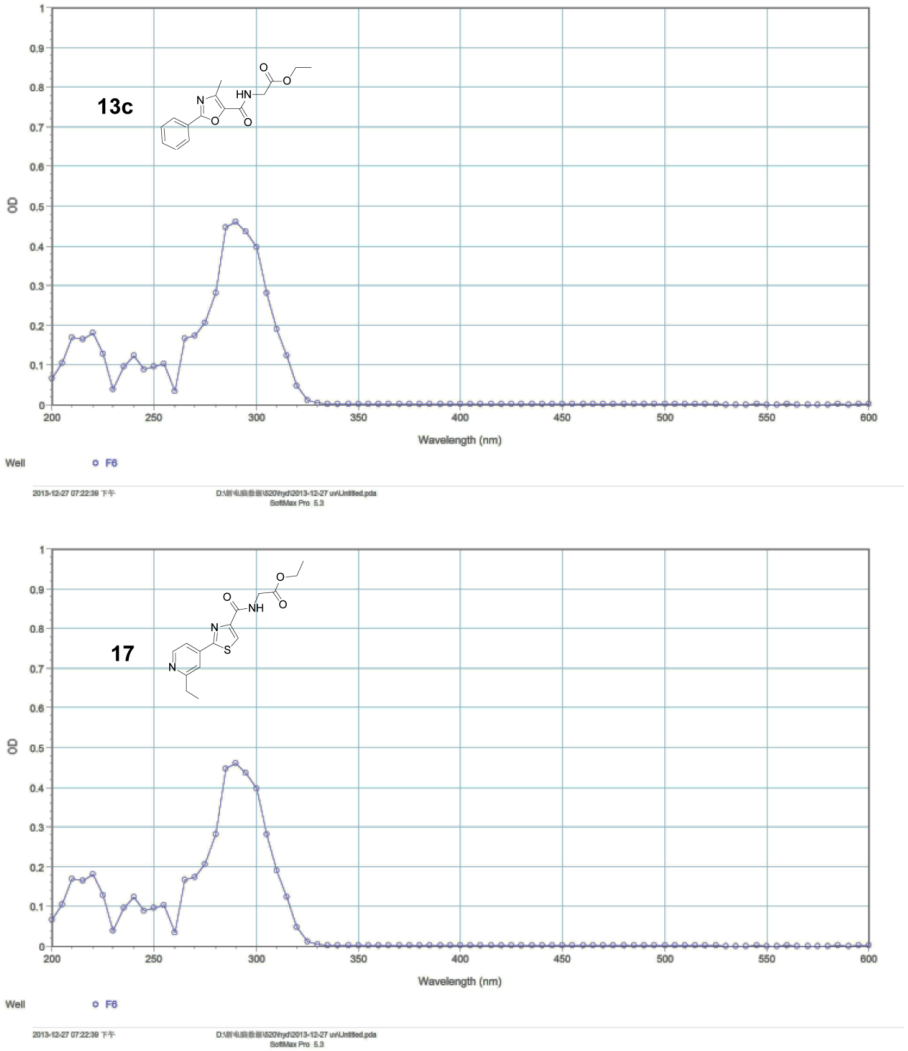

Figure S3B. Ultraviolet absorption spectrum of compound **13c** and **17** (100 μM) under irradiation (Spectra MAX 190, Molecular Devices, CA).

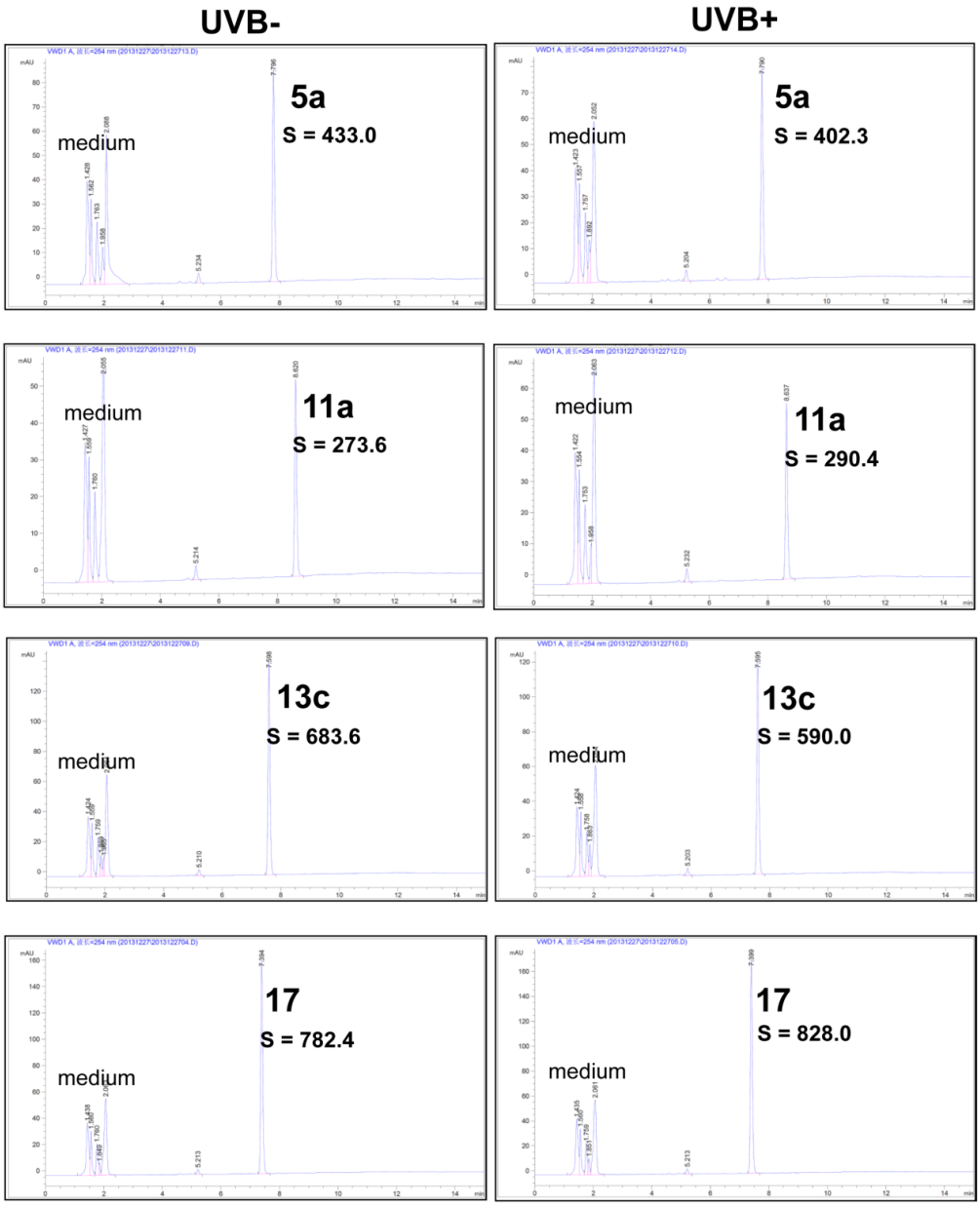

Figure S4. The concentration of compound **5a**, **11a**, **13c** and **17** (100 μM) with (UVB+) or without (UVB-) 30mJ UVB irradiation in 1640 cell culture medium (detected by HPLC).

Reference

[1] T. M. Chiu, C. C. Huang, T. J. Lin, J. Y. Fang, N. L. Wu and C. F. Hung, *J Ethnopharmacol* **2009**, *126*, 108-113.
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[3] X. L. Liang, X. L. Wang, Z. Li, Q. H. Hao and S. Y. Wang, *J Agric Food Chem* **2010**, *58*, 11548-11552.