Supporting information

Rutaecarpine analogues reduce lipid accumulation in adipocytes via inhibiting adipogenesis/lipogenesis with AMPK activation and UPR suppression

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METHODS

Synthesis and Characterization of rutaecarpine analogues (R17 and R18). The synthetic route of the target compounds **R17** and **R18** is shown in Figure 1D. The key intermediate 2-(1H-indol-2-yl)quinazolin-4(3H)-one **4** was prepared according to the Hermecz procedure.¹ Anthranilamide **1** was coupled with triethyl orthopropionate to yield compound **2**. Then it was converted into the brominated compound **3** using bromine in acid condition. The reaction of compound **3** with excess phenylhydrazine afforded the compound **4**, followed by the condensation reaction with polyphosphoric acid producing the intermediate **5**. The formylation reaction with phosphorylchloride in N₂ condition provided the compound **6**. Finally, the target compounds **R17** and **R18** were obtained by directly substitution of compound **6** utilizing 3-(dimethylamino)-1-propylamine or 3-(diethylamino)-1-propylamine.

2-ethylquinazolin-4(3H)-one (2). The solution of anthranilamide **1** (1.36 g, 10 mmol) and triethyl orthopropionate (10 mL) was refluxed for 12 h, cooled to 0 °C, filtrated and washed with ethanol. The crude residue was desiccated in vacuum as a white solid, 83% yield. ¹H NMR (400 MHz, DMSO) δ 12.12 (s, 1H), 8.08 (dd, *J* = 7.9, 1.4 Hz, 1H), 7.83 – 7.73 (m, 1H), 7.60 (d, *J* = 8.1 Hz, 1H), 7.51 – 7.41 (m, 1H), 2.62 (q, *J* = 7.5 Hz, 2H), 1.25 (t, *J* = 7.5 Hz, 3H).

2-(1-bromoethyl)quinazolin-4(3H)-one (3). A solution of Br₂ (1.59 g, 10 mmol) in acetic acid (10 mL) was dropped to a hot solution of intermediate **2** (1.74 g, 10 mmol) and sodium acetate (0.82 g, 10 mmol) in acetic acid (120 mL). The mixture was stirred at 60 °C for 4 h, and the residue was formed after pouring into ice water, filtrated and desiccated in vacuum as white solid, 69% yield. ¹H NMR (400 MHz, DMSO) δ 12.46 (s, 1H), 8.16 – 8.09 (m, 1H), 7.87 – 7.80 (m, 1H), 7.69 (d, *J* = 8.1 Hz, 1H), 7.55 (t, *J* = 7.5 Hz, 1H), 5.10 (q, *J* = 6.8 Hz, 1H), 2.01 (d, *J* = 6.8 Hz, 3H).

2-(1-(2-phenylhydrazono)ethyl)quinazolin-4(3H)-one (4). A solution of intermediate **3** (2.5 g,

10 mmol) and phenylhydrazine (4.32 g, 40 mmol) in ethanol (80 mL) was refluxed for 10 h. The residue was formed after cooling the mixture to room temperature, filtrated and washed with ethanol. The resulting crude product was purified by recrystallization from ethanol to give the desired product **4** as orange solid, 82% yield. ¹H NMR (400 MHz, DMSO) δ 11.46 (s, 1H), 9.86 (s, 1H), 8.13 (dd, J = 7.9, 1.1 Hz, 1H), 7.85 – 7.76 (m, 1H), 7.67 (d, J = 8.1 Hz, 1H), 7.58 (d, J = 8.2 Hz, 2H), 7.48 (t, J = 7.5 Hz, 1H), 7.28 (t, J = 7.8 Hz, 2H), 6.89 (t, J = 7.3 Hz, 1H), 2.35 (s, 3H).

2-(1H-indol-2-yl)quinazolin-4(3H)-one (5). The mixture of intermediate **4** (1.0 g, 3.6 mmol) in PPA (5 mL) was heated at 150 °C for 2 h. After cooling to room temperature, the mixture was diluted with 50 mL water, and then modulated pH value to 7.0 with KOH. The residue was filtrated, washed with water, and desiccated in vacuum to give the desired product **5** as deep green solid, 69% yield. ¹H NMR (400 MHz, DMSO) δ 12.47 (s, 1H), 11.79 (s, 1H), 8.16 (d, J = 7.7 Hz, 1H), 7.85 (t, J = 7.6 Hz, 1H), 7.74 (d, J = 8.1 Hz, 1H), 7.70 – 7.63 (m, 2H), 7.52 (dd, J = 18.3, 7.9 Hz, 2H), 7.23 (t, J = 7.6 Hz, 1H), 7.07 (t, J = 7.4 Hz, 1H).

8-chloro-7,12-dihydroindolo[2',3':3,4]pyrrolo[2,1-*b*]quinazolin-5-one (6). The mixture of intermediate **5** (2.6 g, 10 mmol) in DMF (200 mL) was dropped into the solution of POCl₃ (1.6 g, 11 mmol) in DMF (25 mL) at 0 °C under nitrogen. The mixture was stirred at room temperature for 24 h, and then poured into 300 mL water.² The crude residue was filtrated and purified by flash chromatography on a silica gel to furnish the desired product **6** as yellow solid, 33% yield. ¹H NMR (400 MHz, DMSO) δ 12.77 (s, 1H), 11.22 (s, 1H), 8.36 (d, J = 8.3 Hz, 1H), 8.31 (d, J = 7.9 Hz, 1H), 8.26 – 8.11 (m, 2H), 7.97 – 7.89 (m, 1H), 7.68 (d, J = 8.2 Hz, 1H), 7.36 (t, J = 7.6 Hz, 1H), 7.28 (t, J = 7.5 Hz, 1H), 1.23 (s, 1H).

8-(N³,N³-dimethylaminopropylamino)-7,12-dihydroindolo[2',3':3,4]pyrrolo[2,1-*b*]quinazolin-5-one (R17). The mixture of intermediate **6** (0.31 g, 1 mmol), triethylamine (100 μ L), 3-(dimethylamino)-1-propylamine (650 μ L) in methylbenzene (10 mL) was refluxed for 10 h, cooled to room temperature, extracted with ice water. The organic phase was collected, concentrated, and purified by flash chromatography on a silica gel to furnish the desired product **7** as yellow solid, 56% yield. ¹H NMR (400 MHz, CDCl₃) δ 11.42 (s, 1H), 10.14 (s, 1H), 9.08 (s, 1H), 8.46 (d, J = 8.5 Hz, 1H), 7.76 (d, J = 8.3 Hz, 1H), 7.66 (t, J = 7.6 Hz, 1H), 7.56 (d, J = 8.1 Hz, 1H), 7.38 (dt, J = 6.8, 3.5 Hz, 2H), 7.24 (td, J = 13.0, 6.9 Hz, 2H), 3.75 (dd, J = 10.1, 5.5 Hz, 2H), 2.63 – 2.52 (m, 2H), 2.36 (s, 6H), 1.91 – 1.80 (m, 2H); ¹³C NMR (101 MHz, CDCl₃) δ 191.33, 159.57, 154.52, 149.20, 143.20, 135.06, 132.67, 128.06, 127.03, 126.26, 124.71, 123.15, 122.87, 121.36, 117.46, 114.57, 111.57, 59.37, 45.32, 42.67, 24.22. Purity: 99.7% by HPLC. HRMS (ESI): calcd for (M+H)⁺ (C₂₂H₂₃N₅O) 374.1975, found 374.1972.

8-(N³,N³-diethylaminopropylamino)-7,12-dihydroindolo[2',3':3,4]pyrrolo[2,1-*b*]quinazolin-

5-one (R18). The mixture of intermediate **6** (0.31 g, 1 mmol), triethylamine (100 μ L), 3-(diethylamino)-1-propylamine (650 μ L) in methylbenzene (10 mL) was refluxed for 10 h, cooled to room temperature, extracted with ice water. The organic phase was collected, concentrated, and purified by flash chromatography on a silica gel to furnish the desired product **8** as yellow solid, 51% yield. ^1H NMR (400 MHz, CDCl_3) δ 11.48 (s, 1H), 10.20 (s, 1H), 9.04 (s, 1H), 8.52 (d, $J = 7.5$, 1H), 7.82 (d, $J = 8.3$, 2H), 7.73 (s, 1H), 7.48 (d, $J = 7.5$, 2H), 7.35 – 7.29 (m, 2H), 4.01 – 3.68 (m, 2H), 2.85 – 2.69 (m, 4H), 1.97 (s, 2H), 1.25 (s, 2H), 1.31 – 1.09 (m, 6H); ^{13}C NMR (101 MHz, CDCl_3) δ 191.13, 159.74, 154.60, 149.43, 142.96, 134.85, 132.77, 128.34, 127.16, 126.08, 124.86, 123.40, 122.97, 121.85, 117.60, 114.73, 111.32, 46.95, 42.67, 29.71, 23.90, 11.16. Purity: 98.5% by HPLC. HRMS (ESI): calcd for $(\text{M}+\text{H})^+$ ($\text{C}_{24}\text{H}_{27}\text{N}_5\text{O}$) 402.2294, found 402.2290.

Cell culture and high fructose induced lipid accumulation. The rat hepatoma cell lines FAO were cultured in low glucose DMEM containing 10% (v/v) fetal calf serum. For drug treatment, FAO cells were treated with low glucose medium supplemented with 25 mM high fructose, which increases lipid accumulation by *de novo* lipogenesis. We treated the cells along with the presence or absence of indicated compounds for 24 h and then the triglyceride and protein content were measured.

MTT assay. Cell viability was determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, sigma) assays. Briefly, HEK293 cells were seeded at 10^4 cells/well in 96-well plates. After 24 h, Cells were incubated with each compound for 48h. Supernatant was discarded and cells were incubated with 40 μ L MTT solution (2.5 mg mL^{-1} MTT in DMEM) for 4 h. Then unreacted dye was removed, and then the insoluble formazan crystals were dissolved in DMSO (200 μ L/well) and measured at 570 nm using a microplate reader. The cell viability percentage (%) was expressed as the percentage of cell growth compared to the control, and it was calculated by $A_{570\text{nm}} [\text{compound}]/A_{570\text{nm}}[\text{control}]*100$.

RESULTS

1. Effects of Rut and its analogues R17 and R18 on high fructose-induced lipid accumulation in FAO cells.

Figure S1. Effects of Rut and its analogues (R17 and R18) on high fructose-induced lipid accumulation in FAO cells. FAO cells were cultured in low glucose medium supplemented with 25 mM fructose in the presence or absence of **Rut**, **R17** or **R18** for 24 h and then the lipid accumulation in the cells was determined in terms of triglyceride (TG) content, which was normalized by protein content. To make logarithmic graph, 0 μM was set as 0.0001 μM. Data are expressed as the mean±SE (3 independent experiments were performed). The symbols *, ** and *** indicate significant differences at $P<0.05$ and $P<0.01$ and $P<0.001$ vs vehicle-treated control.

2. Effect of Rut and its analogues (R17 and R18) on cell viability of HEK 293 cells (MTT assay).

Figure S2. Effect of Rut and its analogues (R17 and R18) on cell viability of HEK 293 cells (MTT assay). HEK 293 cells were treated with indicated compounds at 3 μM for 48 h. Data are expressed as the mean±SE (3 independent experiments were performed). NS indicates no significance vs control.

3. Effects of R18 on adipogenesis-related transcription factors and downstream lipogenic enzymes.

Figure S3. Effects of R18 on adipogenesis-related transcription factors and downstream lipogenic enzymes. 3T3-L1 preadipocytes were treated with **R18** for indicated periods during adipocyte differentiation and then harvested. The protein levels were determined by western blot. (A) protein levels of PPAR γ , C/EBP α and SREBP-1c. (3 days of treatment). (B) protein levels of ACC, FAS and SCD-1 (6 days of treatment). All data were normalized to α -tubulin and the fold changes in expression were calculated relative to control. All Data are expressed as the mean $\pm$ SE of 3 independent experiments. The symbols *, ** and *** indicate significant differences at $P<0.05$ and $P<0.01$ and $P<0.001$ vs vehicle-treated control. MDI, a cocktail consists of insulin, dexamethasone and 1-methyl-3-isobutylxanthin.

4. ER stress/UPR pathways are upregulated during adipocytes differentiation in 3T3-L1 cells

Figure S4. ER stress/UPR pathways are upregulated during adipocytes differentiation in 3T3-L1 cells. 3T3-L1 preadipocytes were treated with MDI (insulin, dexamethasone and 1-methyl-3-isobutylxanthin) medium for 6 days during the differentiation. The protein levels and mRNA levels were determined by western blot and RT-PCR. (A) protein levels of UPR markers. (B) XBP1 mRNA splicing.

5. Effects of R18 on the UPR pathway.

Figure S5. Effects of R18 on two branches of the UPR pathway. 3T3-L1 preadipocytes were treated with **R18** at indicated concentrations for 6 days during adipocyte differentiation. The protein levels of UPR markers were examined by western blot. The symbols *, ** and *** indicate significant differences at $P<0.05$ and $P<0.01$ and $P<0.001$ vs vehicle-treated control. MDI, a

cocktail consists of insulin, dexamethasone and 1-methyl-3-isobutylxanthin.

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