Synthesis and Biological Evaluation of the novel Antioxidant Agent Quercetin Derivatives

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Abstract. Three 3-substituted derivatives of quercetin were synthesized in 10-80% overall yields. Their structures were characterized by ^{1}HNMR. Three target compounds (**4a**~**4c**) have not been reported before. The results showed that: pre-treated of PC-12 cells with, cell survival rate was higher than that of quercetin. In this project, the oxidative damage model of PC-12 cells was established and the antioxidant activities of three newly synthesized quercetin derivatives were evaluated. Among them, compound **4b** (30μM) showed the highest antioxidant activity against H_2O_2. Cell viability in pre-treated with or without compound **4b** (30μM) were 53.05% and 10.31%, higher than that of quercetin (47.22%).

Introduction

Oxidative stress has been considered as a major cause of cellular injuries in a variety of clinical abnormalities[1].Flavonoids are polyphenolic compounds that occur ubiquitously in foods of plant origin[2].Quercetin (3,3',4',5,7-pentahydroxy flavone, quercetin) is a grown-derived flavonoids, widely found in herbal and food[3-5]. Quercetin displays antioxidant, anti-inflammatory, anti-metastatic and anticancer activities[6-12].

In this paper, we would like to report the design and a convenient synthetic approach towards a novel series of 3-substituted derivatives of quercetin.The antioxidant activities of target compounds (**4a**~**4c**) were evaluated by MTT assay in the oxidative damage model of PC-12 cells.

EXPERIMENTAL SECTION

Materials and measurements

Used in this article, all reagents and solvents were of analytical grade. The reaction temperature control uses the oil bath temperature modulator. Thin layer chromatography (TLC) with silica gel 60 GF254. Merck precoated plates (0.25 mm) was visualized using UV. 0.1 for flash chromatography on silica gel (particle size 100-200 mesh). ^{1}H spectra were recorded in DMSO on Bruker AM-400 NMR spectrometers using tetramethylsilane (TMS) as an internal standard. The chemical shifts are reported in δ (ppm) relative to tetramethylsilane as internal standard

Synthesis route of Quercetin derivative

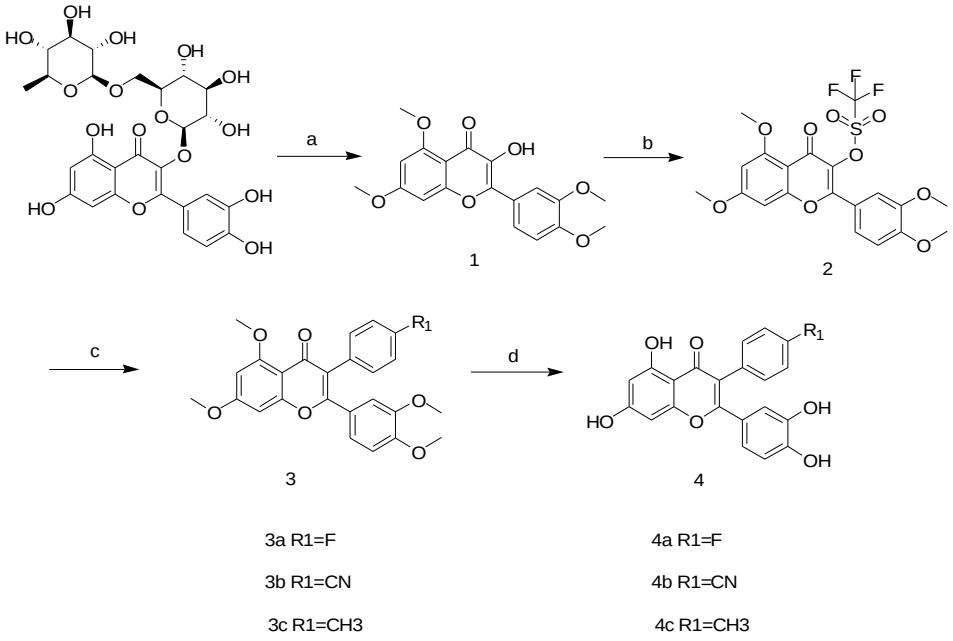

Fig 1 synthesis route of quercetin derivatives

Reagents and conditions: (a) CH_3I, K_2CO_3, DMF, rt. 2%H_2SO_4, 95℃;(b) $PhN(Tf)_2$, K_2CO_3, THF, rt;(c) DMF,CS_2CO_3, $PdCl_2$(dppf), 90℃; (d)DCM,BBr_3, -30 ℃.

Synthesis of 2-(3,4-dimethoxyphenyl)-3-hydroxy-5,7-dimethoxy-4H-chromen-4-one (compound 1)

Rutin (5.00 g) in 30 mL DMF was added K_2CO_3 (5.66 g, 40.95 mmol) and iodomethane (2.5 mL, 40.95 mmol). The reaction mixture was stirred at room temperature for 10 h. Then solvent was removed under reduced pressure. 250 mL 2% H_2SO_4 was added to the residue and then reflux for 3 h. The reaction mixture was filtered and the filter cake was purified by silica gel chromatography using PE:EA (1:1) as the eluent to afford compound **1**.（2.075 g, 71 %）

Synthesis of 2-(3,4-dimethoxyphenyl)-5,7-dimethoxy-4-oxo-4H-chromen-3-yl trifluoromethanesulfonate (compound 2)

Compound **1** (2.79 g) in 10 mL THF was added K_2CO_3 (0.771 g, 5.58 mmol) and N-phenyl-bis trifluoromethanesulfonimide (1.50 g, 4.19 mmol). The reaction mixture was stirred at room temperature for 10 h. Then the reaction was poured into 20 mL of ice water and extracted the mixture with dichloromethane. The reaction mixture was filtered and the filter cake was purified by silica gel chromatography using PE:EA (4:1) as the eluent to afford compound **2**.（1 g, 73%）

Synthesis of 2-(3,4-dimethoxyphenyl)-3-(4-fluorophenyl)-5,7-dimethoxy-4H-chromen-4-one（compound 3a）

Compound **2**（0.2 g，0.4 mmol) in 3 mL DMF was added Cs_2CO_3 (186 mg, 0.56 mmol), 4-Methylphenylboronic acid (77 mg, 0.56 mmol) and $PdCl_2$(dppf) (30 mg, 0.04 mmol). The reaction mixture was stirred at 90℃ for 5 h. Then the reaction was poured into 10 mL of ice water and extracted the mixture with dichloromethane three times. The combined organic layers were dried over Na_2SO_4 to give a residue which was purified by silica gel chromatography using PE:EA(2:1) as the eluent to afford the title compound **3a** (105 mg，59%).

Synthesis of 4-(2-(3,4-dimethoxyphenyl)-5,7-dimethoxy-4-oxo-4H-chromen-3-yl)benzonitrile (compound 3b)

Compound **2**（0.2 g, 0.4 mmol) in 3 mL DMF was added Cs_2CO_3 (186 mg, 0.56 mmol), 4-Cyanophenylboronic acid (84 mg, 0.56 mmol) and $PdCl_2$(dppf) (30 mg, 0.04 mmol). The reaction

mixture was stirred at 90℃ for 5 h. Then the reaction was poured into 10 mL of ice water and extracted the mixture with dichloromethane three times. The combined organic layers were dried over Na_2SO_4 to give a residue which was purified by silica gel chromatography using PE:EA (2:1) as the eluent to afford the title compound **3b** (125 mg，69%).

Synthesis of 2-(3,4-dimethoxyphenyl)-5,7-dimethoxy-3-(p-tolyl)-4H-chromen-4-one (compound 3c)

Compound **2**（0.2 g, 0.4 mmol) in 3 mL DMF was added Cs_2CO_3 (186 mg, 0.56 mmol), 4-Fluorophenylboronic Acid (80 mg ,0.56 mmol) and $PdCl_2$(dppf) (30 mg, 0.04 mmol). The reaction mixture was stirred at 90℃ for 5 h. Then the reaction was poured into 10 mL of ice water and extracted the mixture with dichloromethane three times. The combined organic layers were dried over Na_2SO_4 to give a residue which was purified by silica gel chromatography using PE:EA (2:1) as the eluent to afford the title compound **3c** (130 mg，73%).

Synthesis of 2-(3,4-dihydroxyphenyl)-3-(4-fluorophenyl)-5,7-dihydroxy-4H-chromen-4-one (compound 4a)

To a solution of compound **3a** (40 mg) in DCM (3 ml) was added BBr_3 (0.15 ml) below -30 ℃. After complete addition, the reaction was warmed to room temperature and stirred for 5 h. The reaction mixture was quenched with methanol. Further purification was performed with silica gel chromatography using PE:EA (1:1) as the eluent to afford the title compound **4a** (15 mg，50%).

Synthesis of 4-(2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4-oxo-4H-chromen-3-yl)benzonitrile (compound 4b)

To a solution of compound **3b** (50 mg) in DCM was added BBr_3 (0.15 ml) below -30 ℃. After complete addition, the reaction was warmed to room temperature and stirred for 5 h. The reaction mixture was quenched with methanol. Further purification was performed with silica gel chromatography using PE:EA (1:1) as the eluent to afford the title compound **4b** (5.22 mg，12%).

Synthesis of 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-(p-tolyl)-4H-chromen-4-one (compound 4c)

To a solution of compound **3c** (100 mg) in DCM was added BBr_3 (0.2 ml) below -30 ℃. After complete addition, the reaction was warmed to room temperature and stirred for 5 h. The reaction mixture was quenched with methanol. Further purification was performed with silica gel chromatography using PE:EA (1:1) as the eluent to afford the title compound **4c** (32.32 mg，37%).

Biological assay

In this project, the oxidative damage model of PC-12 cells was established and the antioxidant activities of three newly synthesized quercetin derivatives were evaluated MTT assay. In the oxidative damage model, the cell viability was evaluated by MTT assay to reflect the antioxidant activity against H_2O_2. Brifly, 90 μl of the PC-12 cells were plated in 96-well plates at a density of 5 × 10^4 cells per well and cultured at 37℃ in 5% CO_2 for 16 h. Cells were treated with different concentrations of compounds and incubated at 37 °C for an additional 0.5 h then H_2O_2 (100 μM) was added to the medium for 2 h. MTT assay(the cell viability) was performed using Thermo microplate reader.

RESULTS AND DISCUSSION

Characterize isatin derivatives by ^{1}H NMR.

2-(3,4-dimethoxyphenyl)-5,7-dimethoxy-4-oxo-4H-chromen-3-yl trifluoromethanesulfonate (2)

^{1}H NMR (d_6-DMSO 400 MHz): δ/ppm 3.84 (s, 3H), 3.87 (s, 3H), 3.90 (s, 3H), 3.93 (s, 3H), 6.63(d, *J* = 2.0 Hz, 1H), 6.90(d, *J* = 6.0 Hz, 1H), 7.19 (d, *J* = 8.4 Hz, 1H), 7.44 (d, *J*=2.0 Hz, 1H), 7.49 (t, *J*

= 6.8 Hz, 1H)

2-(3,4-dimethoxyphenyl)-3-(4-fluorophenyl)-5,7-dimethoxy-4H-chromen-4-one (3a)

^{1}H NMR ($CDCl_3$ 400 MHz): δ/ppm 2.32 (s, 3H), 3.48 (s, 3H), 3.80 (s, 3H), 3.91 (s, 3H), 3.93 (s, 3H), 6.37 (d, *J* = 1.6 Hz, 1H), 6.53 (d, *J* = 2.0 Hz, 1H), 6.68 (d, *J* = 2.0 Hz, 1H), 6.80 (d, *J* = 8.4 Hz, 1H), 7.11 (s, 4H) 7.21 (dd, *J* = 2.0, 8.4 Hz, 1H)

4-(2-(3,4-dimethoxyphenyl)-5,7-dimethoxy-4-oxo-4H-chromen-3-yl)benzonitrile (3b)

^{1}H NMR ($CDCl_3$ 400 MHz): δ/ppm 3.60 (s, 3H), 3.89 (s, 3H), 3.93 (s, 3H), 3.94 (s, 3H), 6.40 (d, *J* = 2.0 Hz, 1H), 6.55 (d, *J* = 2.0 Hz, 1H), 6.72 (d, *J* = 1.6 Hz, 1H), 6.78 (d, *J* = 8.4 Hz, 1H), 7.02 (dd, *J* = 2.0, 8.4 Hz, 1H), 7.37 (d, *J* = 8.4 Hz, 2H), 7.59 (d, *J* = 8.0 Hz, 2H)

2-(3,4-dimethoxyphenyl)-5,7-dimethoxy-3-(p-tolyl)-4H-chromen-4-one (3c)

^{1}H NMR ($CDCl_3$ 400 MHz): δ/ppm 3.57 (s, 3H), 3.89 (s, 3H), 3.92 (s, 3H), 3.93 (s, 3H), 6.38 (d, *J* = 2.0 Hz, 1H), 6.54 (d, *J* = 2.4 Hz, 1H), 6.71 (d, *J* = 2.0 Hz, 1H), 6.80 (d, *J* = 8.4 Hz, 1H), 7.00 (t, *J* = 8.8 Hz, 2H), 7.14 (dd, *J* = 2.0, 8.4 Hz, 1H), 7.19-7.23 (m, 2H)

Synthesis of 2-(3,4-dihydroxyphenyl)-3-(4-fluorophenyl)-5,7-dihydroxy-4H-chromen-4-one (4a)

^{1}H NMR (400 MHz, DMSO) δ 13.03 (s, 1H), 10.84 (s, 1H), 9.57 (s, 1H), 9.12 (s, 1H), 7.13 (d, *J* = 7.8 Hz, 2H), 7.05 (d, *J* = 7.5 Hz, 2H), 6.84 (s, 1H), 6.61 (s, 2H), 6.40 (s, 1H), 6.21 (s, 1H), 2.31 (s, 3H).

Synthesis of 4-(2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4-oxo-4H-chromen-3-yl)benzonitrile (4b)

^{1}H NMR (400 MHz, DMSO) δ 12.81 (s, 1H), 10.92 (s, 1H), 9.65 (s, 1H), 9.22 (s, 1H), 7.80 (d, *J* = 7.9 Hz, 2H), 7.40 (d, *J* = 7.9 Hz, 2H), 6.78 (s, 1H), 6.64 (s, 2H), 6.43 (s, 1H), 6.24 (s, 1H).

Synthesis of 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-(p-tolyl)-4H-chromen-4-one (4c)

^{1}H NMR (400 MHz, DMSO) δ 12.95 (s, 1H), 10.86 (s, 1H), 9.59 (s, 1H), 9.16 (s, 1H), 7.26 – 7.13 (m, 4H), 6.81 (s, 1H), 6.63 (s, 2H), 6.41 (d, *J* = 1.9 Hz, 1H), 6.22 (d, *J* = 2.0 Hz, 1H).

Antioxidant activity assay.

The newly prepared 3-substituted derivatives of quercetin were evaluated for their in vitro cytotoxic effects against PC-12 cells by the standard MTT assay with VC as the positive control. The preliminary results were summarized in Table 1.

Table 1 The cell viability of PC-12 treated with H_2O_2 and 3-substituted quercetin derivatives

		H_2O_2					
Compond	DMSO	DMSO	4a	4b	4c	quercetin	VC
Cell viability (%)	100.00	10.31	32.21	53.05	17.27	47.22	65.26

Conclusion

Biological activity test indicated that: compound **4b** (30μM) showed the highest antioxidant activity against H_2O_2. Cell viability in pre-treated with or without compound **4b** (30μM) were 53.05% and 10.31%, higher than that of quercetin (47.22%).

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