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ORIGINAL PAPER

Synthesis and antioxidant activity of some thiazolidin-4-one derivatives

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Abstract 4-Fluorobenzaldehyde was used for the preparation of 2-(4-fluorophenyl)thiazolidin-4-one derivatives which were allowed to react with chloroacetonitrile and acrylonitrile to produce 3-(2-(4-fluorophenyl)-4-oxothiazolidine3-yl)acetonitrile and 3-(2-(4-fluorophenyl)-4-oxothiazoledine-3-yl)propanenitrile. Biological evaluation of some of the compounds showed that many had promising antioxidant activity.

Keywords Thiazolidin-4one · Tetrazoles · Triazoles · Acrylonitrile · Antioxidant

Introduction

Antioxidants are of great interest because of their involvement in important biological and industrial processes. In general, compounds with antioxidant activity have also been found to have anticancer, anti-cardiovascular, anti-inflammation, and many other activities [1–3]. Reactive oxygen species (ROS) and free radicals are considered to be implicated in a variety of pathological events, such as cancer and aging [4–6]. ROS, including superoxide anion, hydrogen peroxide, and hydroxyl radical, are thought to be generated subsequent to the reduction of molecular oxygen in aerobic organisms [7, 8]. Under normal conditions, cells and tissues are protected against ROS by an array of enzyme defense systems, such as superoxide dismutase, catalase, and glutathione peroxidases, in addition to numerous non-enzymatic small molecules distributed widely in the biological system and capable of scavenging free radicals. These molecules include glutathione, α-tocopherol (vitamin E), vitamin C, β-carotene, and selenium [9]. In general, the cell is able to maintain an appropriate balance between oxidants and antioxidants under normal conditions. There has been substantial interest in the chemistry of thiazolidin-4-one ring systems, which is the core structure in a variety of synthetic pharmaceuticals with a broad spectrum of biological activity [10], such as anti-mycobacterial [11], antifungal [12], anti-cancer [13], anti-tuberculosis [14], anticonvulsant [15], anti-edematous [16], anti-diarrhea [17], anti-HIV [18, 19], anti-platelet-activating factor [20], antidiabetic [10], antihistaminic [21], cyclooxygenase inhibitors, lipoxygenase inhibitors [22], and anti-inflammatory and analgesic [23] activity. Therefore, a general, simple, and efficient method for rapid synthesis of thiazolidine-4-ones would be greatly advantageous and warrants further investigations in drug discovery. Following reports of these activities, we decided to synthesize a series of novel thiazolidin-4-one derivatives that contain heterocyclic rings and cyclic and acyclic sugar moieties. As a part of our continuing work in the search for biologically active compounds with nitrogen and sulfur-containing heterocycles, we synthesized substituted thiazoldin-4-ones (Fig. 1).

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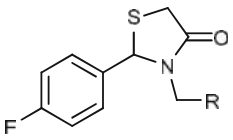
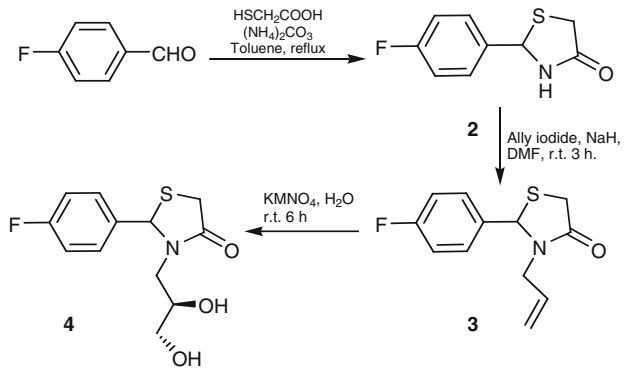


Fig. 1 The substituted thiazoldin-4-ones synthesized (R = aliphatic chain, heterocyclic ring)

Results and discussion

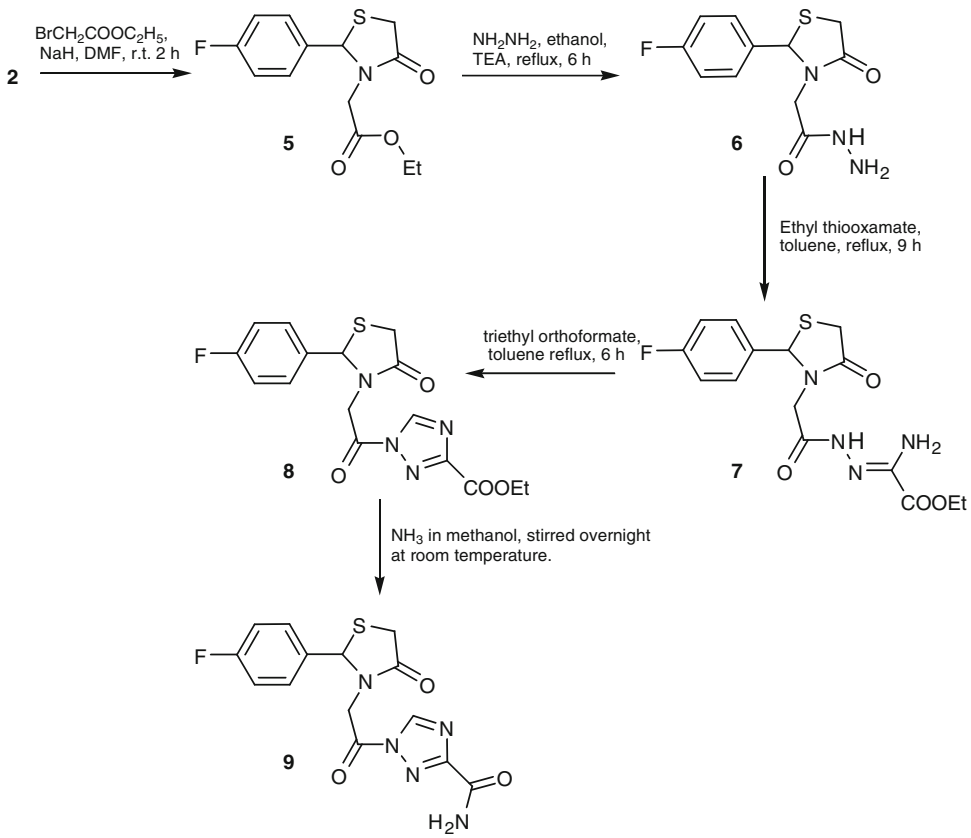
Chemistry

The starting material 2-(4-fluorophenyl)thiazolidine-4-one (**2**) was prepared, in accordance with reported procedures [24], by reaction of 4-fluorobenzaldehyde with thioglycollic acid and ammonium carbonate (molar ratio, 1:1.3:5.2). The cyclocondensation was carried out by heating the reaction mixture under reflux in dry toluene for 18 h, with azeotropic removal of water, to yield **2**, identical with the recently prepared compound [24]. Compound **2** reacted with allyl bromide in dimethylformamide containing sodium hydride via electrophilic substitution to give **3**. The IR spectrum of this reaction product showed the disappearance of an NH group and the ^{1}H NMR spectrum revealed the signals of –NCH_2 and terminal –$CH{=}CH_2$ protons (cf. "Experimental"). Moreover, the mass spectrum of **3**, as typical example, gave $m/z = 237$ which agreed with molecular weight of the molecular formula $C_{12}H_{12}FNOS$ of the assigned structure (cf. Scheme 1). Oxidation of **3** in potassium permanganate afforded the corresponding diol **4**. The IR spectrum showed a band at 3,250–3,300 cm^{-1} of two OH groups, the structure of **4** was established on the basis of data from ^{1}H NMR spectroscopy and elemental analysis (cf. "Experimental"). Moreover, the mass spectrum of **4**, as typical example, gave $m/z = 271$ that agreed with the molecular formula $C_{12}H_{14}FNO_3S$ of the assigned structure (cf. Scheme 1 and "Experimental"). Furthermore, **2** reacted with ethyl bromoacetate in dry acetone containing anhydrous potassium carbonate to give **5**, which is formed by dehydrobromination. The IR spectrum of this reaction product showed the disappearance of the NH group band and, instead, one for the C=O group of the ester, and its ^{1}H NMR spectrum did not reveal the signal of the NH proton and, instead, displayed a triplet and quartet of the $COOCH_2CH_3$ protons of the ester. Considering these results and those from mass spectroscopy and elemental analysis this reaction product was formulated as ethyl 2-(2-(4-fluorophenyl)-4-oxothiazolidin-3-yl)acetate (**5**). Similarly, reaction of **5** with hydrazine hydrate afforded the hydrazide derivatives 2-(2-(4-fluorophenyl)-4-oxothiazoledine-3-yl)acetohydrazide (**6**) (Scheme 2); its structure was elucidated by consideration of results from ^{1}H NMR and elemental analysis (cf. "Experimental"). Moreover, the mass spectrum of **6** gave $m/z = 269.1$ which corresponds to the molecular weight of the molecular formula $C_{11}H_{12}FN_3O_2S$ of the assigned structure (cf. Scheme 2 and "Experimental"). Reaction of **6** with ethylthiooxamate in toluene under reflux gives **7**. The IR spectrum of this compound showed the NH_2 band at 3310 cm^{-1}. Its ^{1}H NMR spectrum revealed the signals of CH_3CH_2 and NH_2 (cf. "Experimental" and Scheme 2). By considering these results, the mass spectrum, and elemental analysis data (cf. "Experimental" and Scheme 2) this reaction product could be formulated as the ethyl-2(2-(2-(4-fluorophenyl)-4-oxothiazolidene3-yl)acetylimino)-2-aminoacetate (**7**). Thus, it was found that **7** reacted with trimethylorthoformate in toluene under reflux to give the corresponding reaction product **8**. The IR spectrum of this reaction product showed no band of an NH_2 group. This reaction product was formulated as ethyl 1-(2-(2-(4-fluorophenyl)-4-oxothiazoldine-3-yl)acetyl)-1H-1,2,4-triazole-3-carboxylate (**8**) on the basis of the above mentioned results, ^{1}H NMR and mass spectroscopy, and elemental analysis (cf. "Experimental" and Scheme 2). Hydrolysis of ester **8** with ammonia in methanol gives the carboxamide **9**. The ^{1}H NMR did not reveal the signals of triplet and quartet of $COOCH_2CH_3$ protons. This reaction product was formulated as 1-(2-(2-(4-fluorophenyl)-4-oxothiazolidine-3-yl) acetyl)-1H-1,2,4-triazole-3-carboxamide (**9**). Compound **2** was cyanomethylated by chloroacetonitrile and sodium hydride in the presence of dimethylformamide to give a reaction product **10** (Scheme 3) formed by dehydrochlorination. The IR of this reaction product contained bands of CN at 2,240 and C=O amide at 1,669 cm^{-1}. On the basis of these results, ^{1}H NMR and mass spectroscopy, and elemental analysis this reaction product was formulated as 3-(2-(4-fluorophenyl)-4-oxothiazolidine3-yl)acetonitrile (**10**). Under similar conditions, compound **2** reacted with acrylonitrile in NaH/DMF to afford the corresponding 3-(2-(4-fluorophenyl)-4-oxothiazoledine-3-yl)propanenitrile (**11**). The structure of **11** was established on the basis of ^{1}H NMR and mass spectroscopy and elemental analysis (cf. "Experimental" and Scheme 3). Compounds **10** and **11**



Scheme 1

Scheme 2

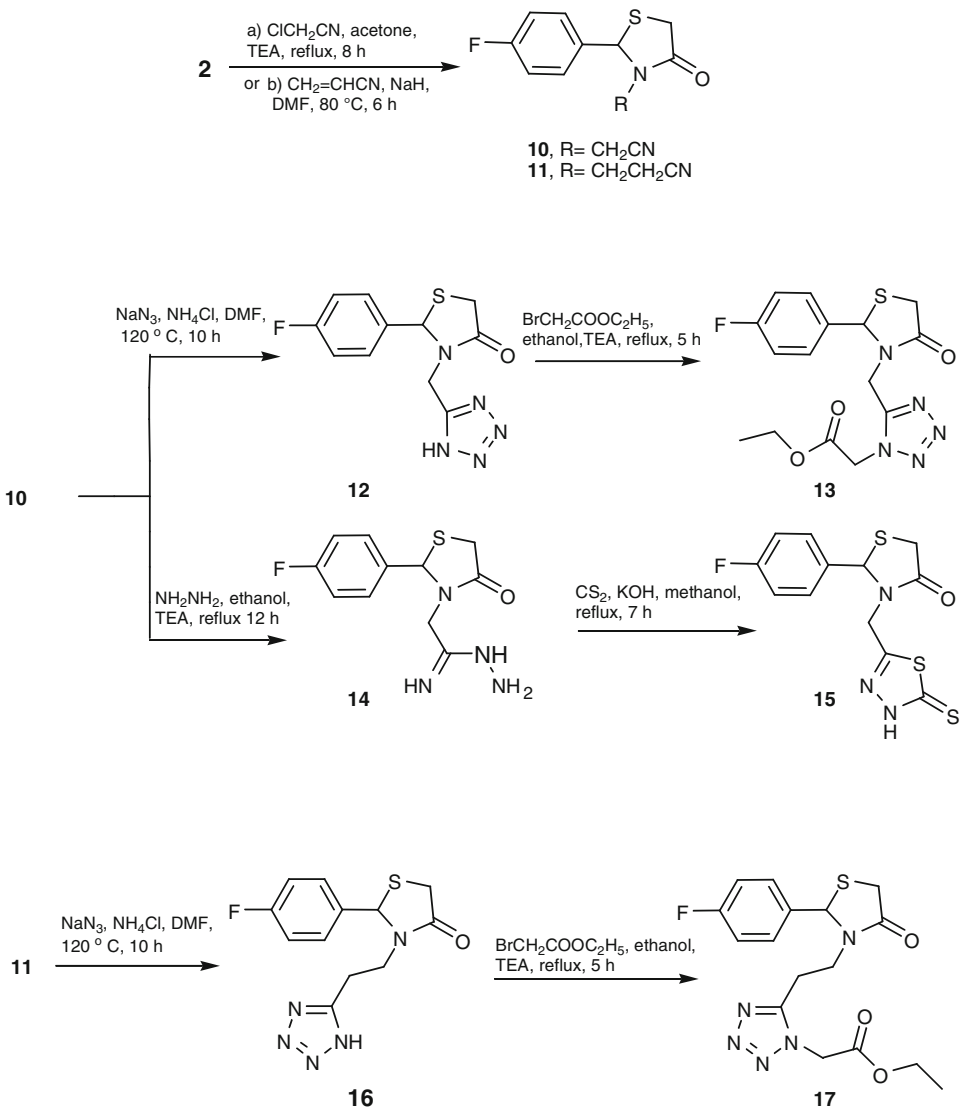


reacted with sodium azide and ammonium chloride in dimethylformamide to give the corresponding 1,2,3,4 tetrazolethiazolid-4-one derivatives **12** and **16** (Scheme 3). The structures of both **12** and **16** were confirmed by results from ^{1}H NMR spectroscopy and elemental analysis (cf. "Experimental"). Moreover, the mass spectra of **12** and **16**, as typical examples, contained peaks at $m/z = 279$ and 293, which agreed with the molecular weights of the molecular formulae $C_{11}H_{10}FN_5OS$ and $C_{12}H_{12}FN_5OS$ of the assigned structures (cf. Scheme 3). The secondary amino group at position-1 of tetrazoles **16** and **12** was submitted to electrophilic substitution with ethyl bromoacetate in boiling ethanol containing a catalytic amount of triethylamine to give the corresponding ethyl 2-(5-((2-(4-fluorophenyl)-4-oxothiazolidin-3-yl)methyl)-1*H*-tetrazol-1-yl) acetate (**13**) and ethyl 2-(5-(2-(2-(4-fluorophenyl)-4-oxothiazolidin-3-yl)ethyl)-1*H*-tetrazol-1-yl)acetate (**17**), respectively. The IR spectra of **13** and **17** showed no bands of NH groups and their ^{1}H NMR spectra revealed the signals of CH_3CH_2 of $COOCH_2CH_3$ protons. Moreover, the mass spectra of **13** and **17**, as typical examples, contained peaks at m/z = 365 and 379 which agreed with the molecular weight of molecular formulae $C_{11}H_{13}FN_4OS$ and $C_{16}H_{18}FN_5O_3S$ of the assigned structures (cf. Scheme 3 and ("Experimental"). Compound **10** was heated under reflux with hydrazine hydrate in ethanol containing a catalytic amount of triethylamine, which, via 1,2-addition of NH_2 of hydrazine hydrate to the CN, yielded the corresponding reaction product amidrazone **14** (Scheme 3). The IR of this reaction product contained no band of CN and, instead, NH_2 and NH bands at 3,380 and 3,190 cm^{-1}. This reaction product could be formulated as 2-(2-(4-fluorophenyl)-4-oxothiazolidine-3-yl) ethaneaminohydrazide (**14**). Cyclization of the amidrazone **14** with carbon disulfide in methanol containing potassium hydroxide yielded 2-(4-fluorophenyl)-3-(4,5-dihydro-5-thioxo-1,3,4-thiadiazole-2-yl)methylthiazolidine-4-one (**15**) (Scheme 3). The structure of **15** was proven by ^{1}H NMR spectroscopy and elemental analysis (cf. "Experimental"). The IR spectrum of this reaction product contained the bands of NH at 3,424, C=O amide at 1,665, and C=S at 1,280 cm^{-1}. Moreover, the mass spectrum of **15** contained a peak at $m/z = 327$, which corresponds to the molecular weight of the molecular formula $C_{12}H_{10}FN_3OS_3$ of the assigned structure.

Biological activity

All the newly synthesized and tested compounds had potent cytotoxic activity against the HT-29 cell line. This cytotoxic activity increased with increasing dose, because we observed that 150 μg cm^{-3} doses induced a higher cell death rate than that induced by 100 μg cm^{-3} and the latter

Scheme 3



induced a higher cell death rate than that induced by a dose of 50 μg cm^{-3}. Cytotoxic activity also increased with increasing contact time between cell line and cytotoxic agents at any fixed dose, so the cell death rate after 48 h was greater than that after 24 h and the cell death rate after 24 h was higher than that after 12 h. The high cell-death activity after 12 h for a small starting dose of 50 μg cm^{-3} indicated potent cytotoxic activity. The most potent compounds were **10**, **9**, **2**, **11**, **12**, **6**, **17**, **7**, **13**, **14**, **3**, **8**, **4**, **15**, and **5** arranged in descending order of activity.

The antioxidant activity of the newly synthesized compounds was investigated by measuring the scavenging effects on the superoxide produced by the HX/XO system, at a dose level of 50 μg cm^{-3}, which is the C50 of vitamin C. The potency relative to vitamin C was determined. It was found that all the compounds had antioxidant activity, as tested by NBT reduction, to different extents and that compounds **3**, **6**, **15**, **13**, **17**, **12**, **9**, **14**, and **7**, which are arranged in descending order, were more potent than vitamin C. It is worth mentioning that compound **3** was nearly three times as active as vitamin C. The other compounds were less active, in the descending order **8**, **10**, **5**, **11**, **16**, **4**, and **2** (Tables 1 and 2).

Conclusion

The presence of extra alicyclic systems attached to the thiazolidone moiety greatly increases cytotoxicity activity, probably because of its DNA-interchelating property. As the number of nitrogen atoms attached to the *N*-allyl moiety increases the cytotoxicity activity decreases. Heterocyclic ring systems attached to the thiazolidone markedly reduce cytotoxic activity. Thiazolidin-4-one is essential for antioxidant activity. *N*-Allyl attached to thiazolidin-4-one moiety sharply increases antioxidant

Table 1 Cytotoxic effect on HT-29 cells at concentrations of 50, 100, and 150 μg cm^{-3}

Compound	Cytotoxic effect/% of 50 μg cm^{-3} in t/h			Cytotoxic effect/% of 100 μg cm^{-3} in t/h			Cytotoxic effect/% of 150 μg cm^{-3} in t/h	
	12	24	48	12	24	48	12	24
2	28	32	33	30	52	55	34	73
3	15	16	19	18	42	52	26	58
4	27	31	40	29	41	52	40	52
5	24	27	39	31	43	57	42	47
6	20	31	35	27	51	66	35	67
7	19	27	32	29	53	66	37	62
8	21	25	31	31	43	57	42	57
9	23	33	42	32	62	77	37	74
10	22	40	44	31	65	74	43	72
11	17	19	22	23	46	62	37	69
12	22	24	30	24	53	62	27	62
13	16	14	22	20	36	55	42	61
14	16	20	35	26	42	47	32	50
15	30	32	40	37	42	56	47	55
16	16	22	27	22	52	68	35	67
17	21	32	25	35	42	53	40	50

Table 2 The scavenging effect of compounds on superoxide at a dose of 50 μg cm^{-3}

Compound	Scavenging effect/% of compounds on superoxide at a dose 50 μg cm^{-3}	Antioxidant potency relative to vitamin C
2	2.12	0.09
3	62.57	2.82
4	7.88	0.35
5	3.81	0.71
6	39.88	1.81
7	25.95	1.17
8	20.65	0.93
9	31.3	1.415
10	13.16	0.599
11	31.96	1.452
12	33.33	1.515
13	26.92	1.22
14	37.98	1.726
15	7.79	0.35
16	32.2	1.463
17	17.99	0.819

activity. As the number of nitrogen atoms attached to the *N*-allyl moiety increases, antioxidant activity decreases. Attachment of other groups to the *N*-allyl greatly reduces antioxidant activity.

Experimental

Melting points were determined on a Gallenkamp melting-point apparatus. Analytical data were obtained from the micro analytical unit, Cairo University, Egypt and results agreed favorably with calculated values. IR spectra (KBr discs) were recorded on a Perkin–Elmer 1430 spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were determined in DMSO-d_6 on a Joel 270 MHz instrument and the chemical shifts were recorded in ppm relative to TMS. The mass spectra were run at 70 eV with a Finnegan SSQ GC–MS spectrometer, using electron-impact (EI) ionization. All reactions were followed by TLC (aluminum foil, silica gel 60 F_{254}, Merck). Merck silica gel (0.040–0.063 mm) was used for column chromatography.

2-(4-Fluorophenyl)thiazolidin-4-one (**2**, C_9H_8FNOS)
A mixture of 12.4 g 4-fluorobenzaldehyde (1 mmol), 11.96 g thioglycollic acid (1.3 mmol), and 49.92 g $(NH4)_2CO_3$ (5.2 mmol) in 150 cm^3 toluene were heated under reflux for 18 h with stirring and collection of the water generated in an azeotropic collector. The solution was then cooled, evaporated under reduced pressure and the oily residue was recrystallized from pet. ether (40–60 °C)–acetone to afford **2** (86%). M.p.: 141–142 °C; ^{1}H NMR (270 MHz, DMSO-d_6): $\delta = 3.64$–3.73 (AB system, $J = 15.4$ Hz, 5-CH_2), 5.82 (s, 2-CH), 7.20–7.40 (m, 4 aromatic protons), 9.00 (s, NH) ppm; IR (KBr): $\bar{\nu} = 3{,}194{,}1{,}664$ cm^{-1}; MS (70 eV): $m/z = 199$ (M + 2) (16.6), 197 (100), 150 (62), 124 (75).

3-Allyl-2-(4-fluorophenyl)thiazolidin-4-one **(3,** $C_{12}H_{12}FNOS$**)**

To a solution of 1.97 g compound **2** (10 mmol) in 20 cm^3 DMF was added 0.48 g NaH (20 mmol) and the mixture was stirred for 30 min, and then 1.68 g allyl iodide (10 mmol) was added. The reaction mixture was stirred at room temperature for 12 h. The mixture was then poured into water and the oil residue obtained was recrystallized from pet. ether (40–60 °C)–acetone to give **3** (75%) as a white solid. M.p.: 186–188 °C; ^{1}H NMR (270 MHz, DMSO-d_6): $\delta = 2.80$ (dd, $J = 5.8$, 9.3 Hz, NC*H*), 3.40–3.53 (AB system, $J = 15.2$ Hz, 5-CH_2), 4.10 (dd, $J = 5.8$, 9.3 Hz, NC*H*) 4.82 (m, $NCH_2CH{=}CH_2$) 5.25 (s, 2-CH), 5.32 (m, $NCH_2CH{=}CH_2$) 6.70–6.90 (m, 4 aromatic protons) ppm; MS (70 eV): $m/z = 237$ (M^+) (80), 191 (75), 162 (100), 139 (70), 109 (84).

2-(4-Fluorophenyl)-3-(2,3-dihydroxypropyl)thiazolidin-4-one **(4,** $C_{12}H_{14}FNO_3S$**)**

To a solution of 9.85 g compound **3** (5.12 mmol) in 50 cm^3 H_2O, stirred for 6 h at room temperature, 18.2 g $KMnO_4$ (10.25 mmol) was added. The solution was extracted with diethyl ether. The ether extracts were combined, dried, and evaporated. The residue was loaded on to a column of silica gel and eluted with pet. ether–ethyl acetate (8:2) to give **4** (78%) as an oily product. ^{1}H NMR (270 MHz, DMSO-d_6): $\delta = 3.75$–3.96 (AB system, $J = 15.5$ Hz, 5-CH_2), 5.67 (s, 2-CH), 6.70–6.90 (m, 4 aromatic protons) ppm; IR (KBr): $\bar{\nu} = 3{,}250$–$3{,}300$ cm^{-1}; MS (70 eV): $m/z = 271$ (M^+) (91), 237 (23) 196 (85), 180(100).

Ethyl 2-(2-(4-fluorophenyl)-4-oxothiazolidin-3-yl) acetate **(5,** $C_{13}H_{14}FNO_3S$**)**

A mixture of 11.6 g compound **2** (0.1 mol), 13.8 g anhydrous potassium carbonate (0.1 mol), and 18 cm^3 ethyl bromoacetate (0.1 mol) in 150 cm^3 dry acetone was heated under reflux for 15 h. The reaction mixture was filtered and the residue was washed with 50 cm^3 acetone. The filtrate was evaporated under reduced pressure and the residue was recrystallized from ethanol to give **5** (91%) as an orange solid. M.p.: 47 °C; ^{1}H NMR (270 MHz, DMSO-d_6): $\delta = 1.32$ (t, CH_3), 3.37 (s, $NCH_2C{=}O$), 3.53–3.72 (AB system, $J = 15.2$ Hz, 5-CH_2), 3.94 (q, CH_2), 5.90 (s, 2-CH), 7.20–7.43 (m, 4 aromatic protons) ppm; MS (70 eV): $m/z = 283$ (M^+) (16), 238 (19), 196 (100), 136 (23), 109 (38).

2-(2-(4-Fluorophenyl)-4-oxothiazolidin-3-yl) acetohydrazide **(6,** $C_{11}H_{12}FN_3O_2S$**)**

Compound **5** (8.1 g, 10 mmol), hydrazine hydrate (20 mmol), and 5 cm^3 triethylamine were heated under reflux in 50 cm^3 absolute ethanol for 7 h. The reaction mixture was cooled and a white precipitate formed. After filtration the solid was recrystallized from ethanol to give **6** (94%) as a white solid. M.p.: 110–112 °C; ^{1}H NMR (270 MHz, DMSO-d_6): $\delta = 3.10$ (br s, NH_2), 3.41 (s, $CH_2C{=}O$), 3.57–3.79 (AB system, $J = 15.1$ Hz, 5-CH_2), 5.91 (s, 2-CH), 7.20–7.42 (m, 4 aromatic protons), 9.00 (s, NH) ppm; MS (70 eV): $m/z = 269.1$ (M^+) (8), 238 (20), 210 (18), 196 (100), 109 (43).

Ethyl 2-(2-(2-(4-fluorophenyl)-4-oxothiazolidin-3-yl) acetylimino)-2-aminoacetate **(7,** $C_{15}H_{17}FN_4O_4S$**)**

A mixture of 3.5 g **6** (13 mmol) and 1.73 g ethyl thiooxamate (13 mmol) in 30 cm^3 toluene was heated under reflux for 3 h. The excess solvent was removed under reduced pressure and the solid product obtained was recrystallized from ethanol to give **7** (74%) as a white powder. M.p.: 207 °C; ^{1}H NMR (270 MHz, DMSO-d_6): $\delta = 1.19$ (t, CH_3), 2.91 (br. s, NH_2), 3.34 (s, $CH_2C{=}O$), 3.73–3.92 (AB system, $J = 15.4$ Hz, 5-CH_2), 4.15 (q, CH_2), 5.93 (s, 2-CH), 7.22–7.47 (m, 4 aromatic protons), 9.85 (s, 1H, NH) ppm; ^{13}C NMR (270 MHz, DMSO-d_6): $\delta = 13.9$, 31.7, 43.4, 58.0, 61.6, 115.8, 129.6, 135.5, 140.2, 161.3, 163.2, 167.7, 171.5 ppm; IR (KBr): $\bar{\nu} = 3{,}310$ cm^{-1}; MS (70 eV): $m/z = 368.1$ (M^+) (8), 351(3), 305(8), 252 (10), 208 (43), 173 (100), 109 (96).

Ethyl-1-(2-(2-(4-fluorophenyl)-4-oxothiazolidin-3-yl) acetyl)-1H-1,2,4-triazole-3-carboxylate **(8,** $C_{16}H_{15}FN_4O_4S$**)**

A mixture of 2.5 g **7** (6.8 mmol) and 2.0 g trimethylorthoformate (20 mmol) in 20 cm^3 toluene was heated under reflux for 5 h. The excess solvent was removed under reduced pressure. The residue obtained was chromatographed on silica gel with pet. ether–AcOEt (2:1) to give **8** (63%) as a white powder. M.p.: 180–183 °C; ^{1}H NMR (270 MHz, DMSO-d_6): $\delta = 1.17$ (t, CH_3), 3.30 (s, $CH_2C{=}O$), 3.75–3.96 (AB system, $J = 15.5$ Hz, 5-CH_2), 4.20 (q, CH_2), 5.84 (s, 2-CH), 7.32–7.57 (m, 4 aromatic protons), 8.85 (s, CH) ppm; ^{13}C NMR (270 MHz, DMSO-d_6): $\delta = 14.2$, 35.4, 45.2, 59.1, 61.2, 115.5,130.4, 134.8, 157.4, 161.2, 161.8, 171.5, 175 ppm; MS (70 eV): $m/z = 378.1$ (M^+) (12), 333 (43), 305(38), 238 (15), 210 (43), 173 (10), 109 (100).

1-(2-(2-(4-Fluorophenyl)-4-oxothiazolidin-3-yl)acetyl)-1H-1,2,4-triazole-3-carboxamide **(9,** $C_{14}H_{12}FN_5O_3S$**)**

To a solution of 2.0 g **8** (5.27 mmol) in 15 cm^3 methanol was added 20 cm^3 NH_3 in methanol and the mixture was stirred overnight at room temperature and concentrated under reduced pressure. The residue obtained was chromatographed on silica gel with pet. ether–AcOEt (1:1) to give **9** (83%) as a white powder. M.p.: 168–170 °C; ^{1}H NMR (270 MHz, DMSO-d_6): $\delta = 3.75$–3.96 (AB system, $J = 15.5$ Hz, 5-CH_2), 4.12 (s, $CH_2C{=}O$), 5.84 (s, 2-CH), 6.20 (br. s, NH_2), 7.22–7.43 (m, 4 aromatic protons), 8.92 (s, CH) ppm; ^{13}C NMR (270 MHz, DMSO-d_6): $\delta = 33.6$,

43.6, 57.8, 115.7, 131.6, 136.3, 158.5, 161.3, 164.3, 169.5, 171.4, 178.3 ppm; MS (70 eV): $m/z = 349.1$ (M^+) (18), 333 (23), 304(58), 238 (35), 109 (100).

2-(2-(4-Fluorophenyl)-4-oxothiazolidin-3-yl) acetonitrile **(10,** $C_{11}H_9FN_2OS$**)**

To a solution of 10 g **2** (56.5 mmol) in 75 cm^3 acetone, 4.25 g chloroacetonitrile (56.6 mmol) was added dropwise and the reaction mixture was heated under reflux for 5 h. The reaction mixture was cooled and colorless crystals were formed. After filtration the solid was recrystallized from ethanol to give **10** (85%) as a pale yellow powder. M.p.: 124–126 °C; ^{1}H NMR (270 MHz, DMSO-d_6): $\delta = 3.75$–3.96 (AB system, $J = 15.5$ Hz, 5-CH_2), 4.33 (s, CH_2CN), 5.90 (s, 2-CH), 7.20–7.43 (m, 4 aromatic protons) ppm; IR (KBr): $\bar{\nu} = 2{,}240$, $1{,}669$ cm^{-1}; MS (70 eV): $m/z = 236$ (M^+) (56), 196 (100), 141 (33), 109 (30).

3-(2-(4-Fluorophenyl)-4-oxothiazolidin-3-yl) propanenitrile **(11,** $C_{12}H_{11}FN_2OS$**)**

To a solution of 1.00 g **2** (10 mmol) in 20 cm^3 DMF was added 0.46 g NaH (20 mmol) and the mixture was stirred for 30 min, and then 0.53 g acrylonitrile (10 mmol) was added. The reaction mixture was stirred at 80 °C for 5 h. The mixture was then poured into water and the oil obtained was recrystallized from pet. ether (40–60°)–acetone to give **11** (90%), as an oily product. ^{1}H NMR (270 MHz, DMSO-d_6): $\delta = 3.75$–3.96 (AB system, $J = 15.5$ Hz, 5-CH_2), 5.84 (s, 2-CH), 7.32–7.57 (m, 4 aromatic protons) ppm; MS (70 eV): $m/z = 250$ (M^+) (100), 236(12), 196 (100), 137 (27), 109 (55).

3-((1H-Tetrazol-5-yl)methyl)-2-(4-fluorophenyl) thiazolidin-4-one **(12,** $C_{11}H_{10}FN_5OS$**)**

A mixture of 4 g **10** (14 mmol), 1.2 g sodium azide (18 mmol) and 0.98 g ammonium chloride (18 mmol) in 10 cm^3 DMF was heated under reflux for 7 h at 125 °C. The solvent was removed under reduced pressure and the residue was dissolved in 100 cm^3 water and carefully acidified with conc. HCl to pH 2. The solution was cooled to 5 °C in an ice bath. Recrystallization from aqueous methanol gave **12** (75%) as a white powder. M.p.: 164–166 °C; ^{1}H NMR (270 MHz, DMSO-d_6): $\delta = 3.75$–3.96 (AB system, $J = 15.5$ Hz, 5-CH_2), 4.33 (s, CH_2), 5.90 (s, 2-CH), 7.20–7.43 (m, 4 aromatic protons) ppm; MS (70 eV): $m/z = 279$ (M^+) (100), 265(19), 251(68), 236 (16), 196 (100), 141 (9), 109 (44).

Ethyl 2-(5-((2-(4-fluorophenyl)-4-oxothiazolidin-3-yl) methyl)-1H-tetrazol-1-yl)acetate **(13,** $C_{15}H_{16}FN_5O_3S$**)**

To a solution of 1.78 g **12** (6.4 mmol) in 50 cm^3 absolute ethanol, 0.8 cm^3 ethyl bromoacetate (6.4 mmol) and a few drops of triethylamine were added. The reaction mixture was heated under reflux on a water bath for 5 h. The reaction mixture was filtered and the residue was washed with 50 cm^3 acetone. The filtrate was evaporated under reduced pressure and the residue was recrystallized from ethanol to give **13** (91%) as an orange solid. M.p.: 47 °C; ^{1}H NMR (270 MHz, DMSO-d_6): $\delta = 1.32$ (t, CH_3), 3.53–3.72 (AB system, $J = 15.2$ Hz, 5-CH_2), 3.91 (q, CH_2), 4.57 (s, $NCH_2C{=}O$), 5.94 (s, 2-CH), 7.20–7.43 (m, 4 aromatic protons) ppm; MS (70 eV): $m/z = 365$ (M^+) (76), 336 (29), 292 (9), 265 (100), 196 (88).

2-(2-(4-Fluorophenyl)-4-oxothiazolidin-3-yl) ethaneiminohydrazide (**14**, $C_{11}H_{13}FN_4OS$)

Compound **10** (3.1 g, 10 mmol), 99% hydrazine hydrate (20 mmol), and 5 cm^3 triethylamine were heated under reflux in 50 cm^3 absolute ethanol for 12 h. The reaction mixture was cooled and a white precipitate formed. After filtration the solid was recrystallized from ethanol to give **14** (74%) as a yellow solid. M.p.: 161–165 °C; ^{1}H NMR (270 MHz, DMSO-d_6): $\delta = 2.90$ (br s, NH_2), 3.21 (s, $CH_2C{=}NH$), 3.31–3.49 (AB system, $J = 15.1$ Hz, 5-CH_2), 5.80 (s, 2-CH), 7.31–7.44 (m, 4 aromatic protons), 9.00 (s, NH) ppm; MS (70 eV): $m/z = 268.08$ (M^+) (28), 237 (12), 210 (33), 196 (80), 140 (13).

2-(4-Fluorophenyl)–3-((4,5-dihydro-5-thioxo-1,3,4-thiadiazol-2-yl)methyl)thiazolidin-4-one **(15,** $C_{12}H_{10}FN_3OS_3$**)**

To a solution of 2 g **14** (7 mmol) in 50 cm^3 absolute methanol 5 cm^3 carbon disulfide were added. The reaction mixture was heated under reflux on a water bath for 5 h and the solvent was then removed under reduced pressure. The residue was crystallized from methanol to give **15** (91%) as an oil. ^{1}H NMR (270 MHz, DMSO-d_6): $\delta = 3.70$–3.92 (AB system, $J = 15.5$ Hz, 5-CH_2), 4.43 (s, CH_2), 5.87(s, 2-CH), 7.19–7.39 (m, 4 aromatic protons) ppm; IR (KBr): $\bar{\nu}$ = 3,424, 1,665, 1,280 cm^{-1}; MS (70 eV): $m/z = 327$ (M+) (10), 297(59), 256(48), 224 (76), 196 (100), 141 (9), 109 (44).

3-(2-(1H-Tetrazol-5-yl)ethyl)-2-(4-fluorophenyl) thiazolidin-4-one **(16,** $C_{12}H_{12}FN_5OS$**)**

A mixture of 4 g **11** (16 mmol), 1.2 g sodium azide (18 mmol), and 0.98 g ammonium chloride (18 mmol) in 10 cm^3 DMF was heated under reflux for 7 h at 125 °C. The solvent was removed under reduced pressure and the residue was dissolved in 100 cm^3 water and carefully acidified with conc. HCl to pH 2. The solution was cooled to 5 °C in an ice bath. Recrystallization from aqueous methanol gave **16** (75%). M.p.: 131–135 °C; ^{1}H NMR (270 MHz, DMSO-d_6): $\delta = 3.75$–3.96 (AB system, $J = 15.5$ Hz, 5-CH_2), 4.33 (s, CH_2), 5.90 (s, 2-H), 7.20–7.43 (m, 4 aromatic protons) ppm; MS (70 eV): $m/z = 293.07$ (M^+) (65), 279(25), 251(20), 238(22), 196 (100), 141 (9), 109 (14).

Ethyl 2-(5-(2-(2-(4-fluorophenyl)-4-oxothiazolidin-3-yl) ethyl)-1H-tetrazol-1-yl)acetate (**17,** $C_{16}H_{18}FN_5O_3S$)
To a solution of 3 g **16** (10 mmol) in 50 cm^3 absolute ethanol 1.5 cm^3 ethyl bromoacetate (12 mmol) and a few drops of triethylamine were added. The reaction mixture was heated under reflux on a water bath for 5 h. The reaction mixture was filtered and the residue was washed with 50 cm^3 acetone. The filtrate was evaporated under reduced pressure and the residue was recrystallized from ethanol to give **17** (73%) as an oil. ^{1}H NMR (270 MHz, DMSO-d_6): $\delta = 1.22$ (t, CH_3), 3.53–3.72 (AB system, $J = 15.2$ Hz, 5-CH_2), 3.91 (q, CH_2), 4.57 (s, $NCH_2C{=}O$), 5.94 (s, 1H, 2CH), 7.20–7.43 (m, 4 aromatic protons) ppm; MS (70 eV): $m/z = 379.1$ (M^+) (36), 350 (79), 306 (13), 293 (45), 279(15), 251(50), 238(86), 196 (100), 141 (11), 109 (44).

Screening of the compounds as superoxide radical scavengers

Superoxide radicals were generated by xanthine/xanth. oxidase (XO) and measured by the nitroblue tetrazolium (NBT) reduction method. A test sample was mixed with 100 mM phosphate buffer solution (pH 7.0) containing XO (1.65×10^{-2} U cm^{-3}) and NBT (133 μM) at 25 °C in 96-well flat-bottomed microassay plates. The measurement was started by adding xanthine (164 μM). Production of superoxide radical was followed spectrophotometrically at 560 nm at 25 °C for 10 min. Superoxide scavenging activity was calculated according to:

$$\text{Superoxide scavenging activity } (\%) = \text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}} \times 100/\text{Absorbance}_{\text{control}},$$

where $\text{Absorbance}_{\text{control}}$ and $\text{Absorbance}_{\text{sample}}$ represent the absorbance in the absence and presence of samples [25].

Cytotoxicity in HT-29 cells

Cell cultures

HT-29 cells were obtained from the Korean Cell Line Bank (KCLB, Seoul, Korea) and cultured in DMEM supplemented with 10% FBS, 100 U cm^{-3} penicillin, and 100 μg cm^{-3} streptomycin at 37 °C under 5% CO_2 atmosphere. Cells (10^6 cm^{-3}) were cultured in 35 mm culture dishes and in 96-well plates (Becton Dickinson Labware). The final volumes of culture media were 2 cm^3 for the 35 mm culture dish and 100 mm^3 for each well on the 96-well plate.

Neutral red assay

All experiments were performed on the cultured HT-29 cells. Briefly, cells ($10^6/cm^3$) were exposed to 100 mU cm^{-3} in culture media containing 0.5% D-glucose without fetal bovine serum and then incubated in the presence of different concentrations of the thiazoldinone derivatives. After two days of culture, a neutral red uptake assay was performed according to the method of Wadsworth and Koop [26]. Cells (10^6 cm^{-3}) were cultured in a 96-well plate for 12 and 24 h. Following the various treatments, the medium was removed and the cells were incubated in 100 mm^3 new medium containing 10 μg cm^{-3} neutral red for 90 min at 37 °C. After neutral red treatment, the medium was removed, and the wells were washed three times with 100 mm^3 PBS. One hundred microliters of 50% ethanol containing 50 mM sodium citrate (pH 4.2) was added into each well on the 96-well multiple plate. After 20 min, the absorbance was measured at 510 nm using a Spectra Count (Packard Instrument, Downers Grove, USA) ELISA reader.

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