Synthesis and Biological Activities of Some New Thiazolidine Derivatives Containing Pyrazole Ring System

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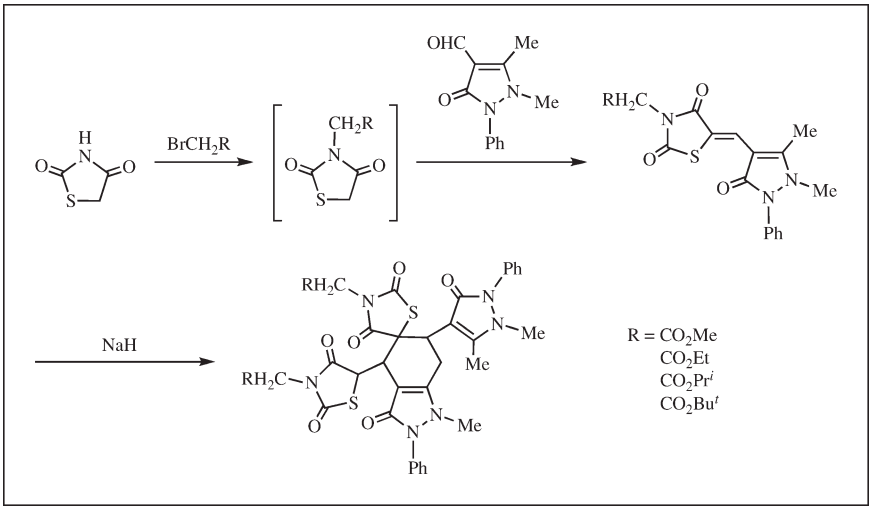

As a part of systematic investigation of synthesis and biologically active compounds of thiazolidine (TZD) derivatives containing pyrazole ring system, several new pyrazole–TZD derivatives **8a**–**d** and **9a**–**d** have been synthesized. Compounds **8a**–**d** were prepared from *N*-substituted TZDs **6a**–**d** and 1*H*-pyrazole-4-carboxaldehyde **7** by Knoevenagel-type reaction. Treatment of **8a**–**d** with sodium hydride at room temperature caused dimerization reaction to afford the corresponding spirocompounds **9a**–**d**. All the synthesized compounds were characterized by spectroscopic analysis. *In vitro*, the synthesized compounds **8a**–**d** and **9a**–**d** were tested for their growth inhibitory activity in A549 lung cancer, B16F10 murine melanoma, and HeLa human uterine carcinoma cells and for their differentiation of 3T3-L1 preadipocytes to adipocytes. The results showed that compound **8c** possessed growth inhibitory effect of B16F10 cells (IC_{50} = 27 μM) and compounds **9c,d** had induction effect on the differentiation of 3T3-L1 preadipocytes.

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INTRODUCTION

The thiazolidine (TZD) and pyrazole derivatives are the most extensively investigated classes of compounds. TZDs classes of insulin sensitizers, synthesized in early 1980s [1], were later found to mediate hypoglycemic effect through peroxisome proliferator-activated receptor γ (PPARγ) [2]. Currently, rosiglitazone and pioglitazone are clinically available TZDs for the treatment of type 2 diabetes. Furthermore, TZD derivatives have attracted very significant biochemical interest, owing to the presence of the TZD moiety in the structures of several naturally occurring molecules with important pharmacological properties such as antidiabetic, antibiotic, and antifungal activities [3–7]. In this context, the synthesis of TZD derivatives continues to attract attention and provides an interesting challenge [8–13].

On the other hand, compounds containing the pyrazole ring system are known to possess pharmacological activities such as analgesic, antidepressant, antibacterial, plant growth regulatory, anti-inflammatory, and antihyperglycemic activities [14–19]. Therefore, the development of improved methods for the synthesis of substituted pyrazole derivatives has acquired relevance to current research [20–23].

Based on these properties, it can be reasonably supposed that the development of synthetic strategies for new TZD derivatives containing the pyrazole ring system might provide additional lead molecules for drug discovery. The molecular hybridization assumption encouraged us to design a specific programme aimed at synthesizing several new derivatives of these ring system. To the best of our knowledge, there are relatively

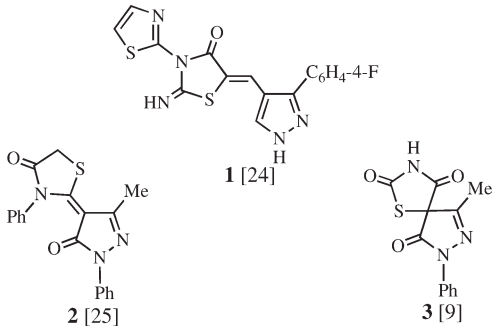


Figure 1. Structures of reported TZD derivatives containing pyrazole ring system.

few methods on the literature describing the preparation of pyrazole–TZD derivatives **1** [24], **2** [25], and **3** [9] (Fig. 1), even though both TZDs [1,2,26–28] and pyrazoles [29–33] have antitumor and antidiabetical activities. For these reasons, we have been interested in the preparation of various types of pyrazole–TZD derivatives to evaluate their biological activities and now report the results of our investigation, the growth inhibitory and differentiation-inducing activities of some new pyrazole–TZD derivatives in tumor and preadipose cells.

RESULTS AND DISCUSSION

Initially, the synthesis of *N*-substituted TZDs **6a–d** has been accomplished as outlined in Scheme 1. In fact, 2,4-thiazolidinedione (**4**) reacted smoothly with α-bromo esters **5a–d** in the presence of sodium hydride in refluxing tetrahydrofuran to give the corresponding **6a–d** [3,5,34–37] in good yields. The results are summarized in Table 1 (entries 1–4). Elemental analyses and spectral data of **6a–d** are consistent with the assigned structures (see Experimental section).

In the next step, a Knoevenagel-type condensation [3,5,7,38,39] of *N*-substituted TZDs **6a–d** and 1*H*-pyrazole-4-carboxaldehyde **7** was examined. When a mixture

Scheme 1

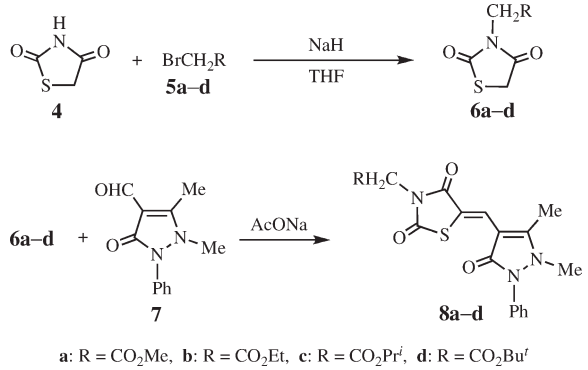


Table 1

Synthesis of compounds **6a–d** and **8a–d** according to Scheme 1.

Entry	Product	Yield (%)
1	**6a** [3,5,37]	90
2	**6b** [34,36]	96
3	**6c**	99
4	**6d** [5]	78
5	**8a**	66
6	**8b**	65
7	**8c**	64
8	**8d**	42

of **6a–d**, **7**, and sodium acetate in the absence of solvent was stirred at 140°C for 30 min, the expected pyrazole–TZD derivatives **8a–d** were obtained in moderate yields (Scheme 1 and entries 5–8 in Table 1). The infrared (IR) spectra of **8a–d** display bands in the range of 1651–1753 cm^{-1} due to four carbonyl groups. The ^1H NMR spectra of **8a–d** in deuteriochloroform exhibit a two-proton singlet near δ 4.4 attributable to the methylene protons and a one-proton singlet near δ 7.6 due to the olefin proton. The ^{13}C NMR spectra of **8a–d** show a signal near δ 42 due to the methylene carbon, a signal near δ 124 due to the olefin carbon, and four signals in the range of δ 162–170 due to the carbonyl carbons. Interestingly, the NMR spectra indicated that **8a–d** existed as a geometrical single isomer of *Z* configuration. It seems that the exocyclic double bond is exclusively in the *Z* configuration because of the high degree of thermodynamic stability of this isomer [28,40]. Elemental analyses and spectral data of **8a–d** are consistent with the proposed structures (see Experimental section).

Based on these results, we have tried to directly construct pyrazole–TZD derivatives **8a–d** from **4**, α-bromo esters **5a–d** and 1*H*-pyrazole-4-carboxaldehyde **7** in a one-pot process. The results are summarized in Table 2. Thus, after a mixture of **4** and 1.0 equivalent of **5a–d** in the presence of 1.0 equivalent of sodium hydride in tetrahydrofuran was refluxed for 3 h, the reaction mixture was treated with 1.0 equivalent of 1*H*-pyrazole-4-carboxaldehyde **7** in the presence of 1.0 equivalent of

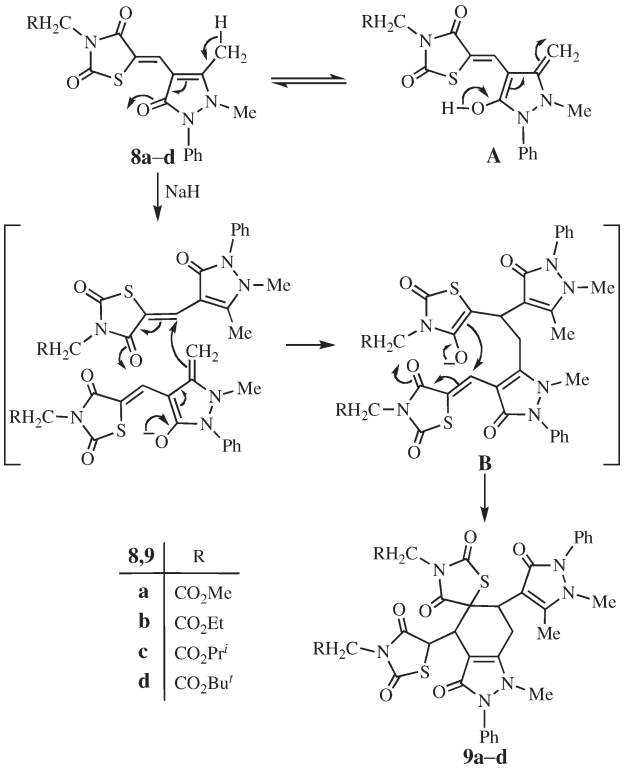
Table 2

One-pot synthesis of pyrazole–thiazolidine derivatives **8a–d**.

4 $\xrightarrow[2)\ \mathbf{7},\ AcONa]{1)\ NaH,\ \mathbf{5a-d}.\ THF}$ **8a – d**

Entry	Substrate	α–Bromo Esters	Product	Yield (%)
1	**4**	**5a**	**8a**	54
2	**4**	**5b**	**8b**	52
3	**4**	**5c**	**8c**	60
4	**4**	**5d**	**8d**	41

Scheme 2



8,9	R
a	CO_2Me
b	CO_2Et
c	CO_2Pr^i
d	CO_2Bu^t

sodium acetate at 140°C for 30 min without solvent, the desired pyrazole–TZD derivatives **8a–d** were obtained in moderate yields (entries 1–4).

Interestingly, we found the reaction condition under which the spirocompounds **9a–d** could be isolated in the presence of sodium hydride. Subsequently, treatment of pyrazole–TZD derivatives **8a–d** with sodium hydride in *N*,*N*-dimethylformamide at room temperature caused dimerization reaction to give the corresponding spirocompounds **9a–d** in moderate yields (Scheme 2 and Table 3). The IR spectra of **9a–d** display bands in the range of 1649−1755 cm^{-1} due to eight carbonyl groups. The ^1H NMR spectra of **9a–d** in deuteriochloroform exhibit a three-proton singlet near δ 2.2 attributable to the methyl protons and two three-proton singlets near δ 3.1 due to two *N*-methyl groups. The ^{13}C NMR spectra of **9a–d** show a signal near δ 12 due to the methyl carbon, two signals near δ 35 due to two *N*-methyl carbons, three signals in the range of δ 39–50 due to the methine carbon, a signal near δ 70 due to the spirocarbon, and eight signals in the range of δ 163–175 due to the carbonyl carbon. By comparison of the NMR, mass spectra, and elemental analyses of **9a–d**, it seems that the structural assignments given to these compounds are correct (see Experimental section). Although there are several possible stereoisomers **9a–d**, the configuration of isolated compounds was not confirmed by NMR spectroscopy.

The formation of the spirocompounds **9a–d** could be explained by possible mechanism presented in Scheme 2. An isomerization of the keto form **8a–d** to the enol form **A** would easily occur in the presence of sodium hydride. Thus, the reaction of **8a–d** with sodium hydride probably causes the conjugate addition of **A** to **8a–d** to give the intermediate Michael adducts **B**, which could then undergo intramolecular conjugate addition to afford the corresponding spirocompounds **9a–d**. In this reaction, the intermediate Michael adducts **B** were not be detected. It makes us believe that the intermolecular/intramolecular Michael addition reaction, namely dimerization reaction of **8a–d** can readily be promoted by using a sodium hydride/*N*,*N*-dimethylformamide system.

To elucidate *in vitro* biological activity of **8a–d** and **9a–d**, their antitumor activity was measured in A549 lung cancer, B16F10 murine melanoma, and HeLa human uterine carcinoma cells using MTT assay [41,42]. The 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) cell proliferation assay has been widely accepted as a reliable way to measure the cell proliferation rate. Although the data obtained by MTT assay showed that almost the synthesized compounds had no inhibitory effects on the growth of tumor cells in dosage-dependent manners, compound **8c** was the most potent compound in this series, having a growth inhibitory property (IC_{50}) value of 27 μM in suppressing B16F10 cell growth.

Furthermore, the differentiation-inducing effect of compounds **8a–d** and **9a–d** on preadipose cells was also investigated using Oil Red O-staining to assess triglyceride accumulation in 3T3-L1 cells [43], which were cultured with compounds **8a–d** and **9a–d**. The results are shown in Figure 2. Pioglitazone, as a full PPARγ agonist, was used as a positive control and the absorbance value in its group was significantly larger than that in the induced medium. It is worth noting that compounds **9c,d** were shown to possess high differentiation-inducing activity in this pyrazole-containing TZD series.

In conclusion, we have described a facile approach to prepare pyrazole–TZD derivatives **8a–d** by a Knoevenagel-type condensation of *N*-substituted TZDs **6a–d** and 1*H*-pyrazole-4-carboxaldehyde **7**. Furthermore, we have developed a novel method for the construction of spirocompounds **9a–d**, proceeding by a dimerization reaction

Table 3

Reaction of compounds **8a–d** with sodium hydride.

Entry	Substrate	Product	Yield (%)
1	**8a**	**9a**	50
2	**8b**	**9b**	28
3	**8c**	**9c**	41
4	**8d**	**9d**	48

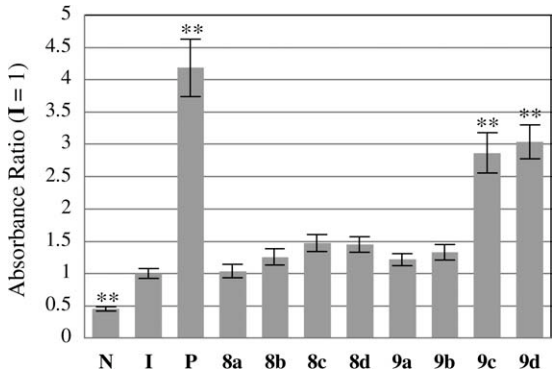


Figure 2. Induction effect of compounds **8a–d** and **9a–d** on differentiation of 3T3-L1 to adipocytes. The data were presented as mean ± SE ($n = 8$). **, $p < 0.01$, when compared with induced medium; **N**, noninduced medium; **I**, induced medium; **P**, pioglitazone.

when **8a–d** were treated with sodium hydride. Interestingly, we found that compound **8c** could suppress B16F10 cancer cell growth. **8c** was the most effective molecule in inhibiting B16F10 cell growth and might perform its action through including apoptosis. In addition, compounds **9c,d** showed high differentiation-inducing activity in 3T3-L1 preadipocytes *in vitro*, which was similar in effect to that of pioglitazone as a PPARγ agonist. Our results suggest that these new pyrazole–TZD derivatives might play a role *in vivo* as anticancer or antidiabetic agents. Functionalized pyrazole–TZD derivatives are important synthons in organic synthesis and for the preparation of biologically active compounds with interest in medicinal chemistry. Further studies on the synthesis of new substituted pyrazole–TZD derivatives are under way.

EXPERIMENTAL

All melting points are uncorrected. The IR spectra were recorded on a JASCO Fourier transform infrared spectroscopy (FT/IR)-4100 spectrometer. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a JEOL JNM-A500 spectrometer at 500 and 125 MHz, respectively. The ^{1}H and ^{13}C chemical shifts (δ) are reported in parts per million (ppm) relative to tetramethylsilane as internal standard. The positive fast atom bombardment (FAB) mass spectra were obtained on a JEOL JMS-700T spectrometer. The elemental analyses were performed on a YANACO MT-6 CHN analyzer.

General procedure for the preparation of *N*-substituted TZDs 6a–d from 4 and α-bromo esters 5a–d. To an ice-cooled and stirred solution of **4** (1.17 g, 10 mmol) in tetrahydrofuran (30 mL) was added 60% sodium hydride (0.44 g, 11 mmol). The stirring was continued at room temperature until evolution of gas ceased. To the obtained solution was added methyl bromoacetate (**5a**) (2.29 g, 15 mmol), ethyl bromoacetate (**5b**) (2.51 g, 15 mmol), isopropyl bromoacetate (**5c**) (2.72 g, 15 mmol), or *tert*-butyl bromoacetate (**5d**) (2.93 g, 15 mmol) with stirring and ice cooling, and then the mixture was refluxed for 3 h. After removal of the solvent *in vacuo*, cold water was added to the residue. The resulting mixture was extracted with ethyl acetate (60 mL). The extract was dried over anhydrous sodium sulfate and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel with chloroform as the eluent to give **6a** [3,5,35] (1.71 g, 90%), **6b** [32,34] (1.95 g, 96%), **6c** (2.14 g, 99%), and **6d** [5] (1.81 g, 78%).

Isopropyl (2,4-dioxo-3-thiazolidine)acetate (6c). This compound was obtained as pale yellow oil; IR (neat): ν 1742, 1690 cm^{-1} (C=O); ^{1}H NMR (deuteriochloroform): δ 1.26 (d, $J = 6.1$ Hz, 6H, $CO_2CH\mathit{Me}_2$), 4.03 (s, 2H, TZD 5-H), 4.30 (s, 2H, α-CH_2), 5.06 ppm (sep, $J = 6.1$ Hz, 1H, $CO_2C\mathit{H}Me_2$); ^{13}C NMR (deuteriochloroform): δ 21.7 ($CO_2CH\mathit{Me}_2$), 33.8 (TZD C-5), 42.4 (α-CH_2), 70.1 ($CO_2\mathit{C}HMe_2$), 165.6 ($\mathit{C}O_2CHMe_2$), 170.1 (TZD C-4), 171.0 ppm (TZD C-2); ms: *m/z* 218 $[M+H]^+$. Anal. Calcd. for $C_8H_{11}NO_4S$: C, 44.23; H, 5.10; N, 6.45. Found: C, 44.16; H, 5.12; N, 6.19.

General procedure for the preparation of pyrazole–TZDs 8a–d from 6a–d and 7. A mixture of **6a–d** (10 mmol), **7** (2.16 g, 10 mmol), and sodium acetate (0.82 g, 10 mmol) was stirred at 140°C for 30 min. The reaction mixture was purified by column chromatography on silica gel with chloroform as the eluent to afford **8a–d**.

Methyl (Z)-{5-[(1,2-dihydro-1,5-dimethyl-3-oxo-2-phenyl-3H-pyrazol-4-yl)methylene]-2,4-dioxo-3-thiazolidine}acetate (8a). This compound was obtained as pale yellow needles, mp 201–203°C (chloroform–petroleum ether); IR (potassium bromide): ν 1742, 1716, 1670 cm^{-1} (C=O); ^{1}H NMR (deuteriochloroform): δ 2.40 (s, 3H, pyrazole 5-Me), 3.30 (s, 3H, pyrazole 1-Me), 3.75 (s, 3H, CO_2Me), 4.43 (s, 2H, NCH_2), 7.31–7.33 (m, 2H, Ph-H), 7.38–7.41 (m, 1H, Ph-H), 7.48–7.51 (m, 2H, Ph-H), 7.59 ppm (s, 1H, C=C*H*—); ^{13}C NMR (deuteriochloroform): δ 10.9 (pyrazole 5-Me), 34.7 (pyrazole 1-Me), 41.7 (NCH_2), 52.6 ($CO_2\mathit{Me}$), 102.7 (pyrazole C-4), 117.0 (TZD C-5), 124.4 (C=*C*H—), 126.1, 128.5, 129.6, 133.5 (Ph-C), 153.4 (pyrazole C-5), 162.3 (pyrazole C-3), 166.8, 167.1 (2 × C=O), 169.8 ppm ($\mathit{C}O_2Me$); ms: *m/z* 388 $[M+H]^+$. Anal. Calcd. for $C_{18}H_{17}N_3O_5S$: C, 55.80; H, 4.42; N, 10.85. Found: C, 55.52; H, 4.49; N, 10.75.

Ethyl (Z)-{5-[(1,2-dihydro-1,5-dimethyl-3-oxo-2-phenyl-3H-pyrazol-4-yl)methylene]-2,4-dioxo-3-thiazolidine}acetate (8b). This compound was obtained as pale yellow needles, mp 165–167°C (acetone–petroleum ether); IR (potassium bromide): ν 1747, 1727, 1672,

1655 cm^{-1} (C=O); ^1H NMR (deuteriochloroform): δ 1.27 (t, J = 7.0 Hz, 3H, CO_2CH_2Me), 2.40 (s, 3H, pyrazole 5-Me), 3.30 (s, 3H, pyrazole 1-Me), 4.21 (q, J = 7.0 Hz, 2H, CO_2CH_2Me), 4.41 (s, 2H, NCH_2), 7.31–7.33 (m, 2H, Ph-H), 7.38–7.41 (m, 1H, Ph-H), 7.48–7.51 (m, 2H, Ph-H), 7.60 ppm (s, 1H, C=CH—); ^{13}C NMR (deuteriochloroform): δ 11.0 (pyrazole 5-Me), 14.1 (CO_2CH_2Me), 34.7 (pyrazole 1-Me), 41.9 (NCH_2), 61.8 (CO_2CH_2Me), 102.8 (pyrazole C-4), 117.2 (TZD C-5), 124.2 (C=CH—), 126.1, 128.5, 129.6, 133.5 (Ph-C), 153.5 (pyrazole C-5), 162.4 (pyrazole C-3), 166.6 (CO_2CH_2Me), 166.9, 169.8 ppm (2 × C=O); ms: m/z 402 $[M+H]^+$. Anal. Calcd. for $C_{19}H_{19}N_3O_5S$: C, 56.85; H, 4.77; N, 10.47. Found: C, 56.87; H, 4.79; N, 10.47.

Isopropyl (Z)-{5-[(1,2-dihydro-1,5-dimethyl-3-oxo-2-phenyl-3H-pyrazol-4-yl)methylene]-2,4-dioxo-3-thiazolidine}acetate (8c). This compound was obtained as pale yellow needles, mp 202–204°C (chloroform–petroleum ether); IR (potassium bromide): ν 1753, 1719, 1671, 1651 cm^{-1} (C=O); ^1H NMR (deuteriochloroform): δ 1.25 (d, J = 6.4 Hz, 6H, CO_2CHMe_2), 2.39 (s, 3H, pyrazole 5-Me), 3.30 (s, 3H, pyrazole 1-Me), 4.38 (s, 2H, NCH_2), 5.06 (sep, J = 6.4 Hz, 1H, CO_2CHMe_2), 7.30–7.32 (m, 2H, Ph-H), 7.37–7.41 (m, 1H, Ph-H), 7.47–7.51 (m, 2H, Ph-H), 7.59 ppm (s, 1H, C=CH—); ^{13}C NMR (deuteriochloroform): δ 10.9 (pyrazole 5-Me), 21.7 (CO_2CHMe_2), 34.7 (pyrazole 1-Me), 42.1 (NCH_2), 69.7 (CO_2CHMe_2), 102.7 (pyrazole C-4), 117.1 (TZD C-5), 124.2 (C=CH—), 126.1, 128.5, 129.6, 133.5 (Ph-C), 153.5 (pyrazole C-5), 162.3 (pyrazole C-3), 166.1 (CO_2CHMe_2), 166.9, 169.8 ppm (2 × C=O); ms: m/z 416 $[M+H]^+$. Anal. Calcd. for $C_{20}H_{21}N_3O_5S$: C, 57.82; H, 5.09; N, 10.11. Found: C, 57.65; H, 5.15; N, 10.06.

***tert*-Butyl (Z)-{5-[(1,2-dihydro-1,5-dimethyl-3-oxo-2-phenyl-3H-pyrazol-4-yl)methylene]-2,4-dioxo-3-thiazolidine}acetate (8d).** This compound was obtained as pale yellow needles, mp 191–193°C dec. (chloroform–petroleum ether); IR (potassium bromide): ν 1739, 1719, 1671 cm^{-1} (C=O); ^1H NMR (deuteriochloroform): δ 1.46 (s, 9H, CO_2CMe_3), 2.40 (s, 3H, pyrazole 5-Me), 3.30 (s, 3H, pyrazole 1-Me), 4.32 (s, 2H, NCH_2), 7.31–7.33 (m, 2H, Ph-H), 7.37–7.41 (m, 1H, Ph-H), 7.48–7.51 (m, 2H, Ph-H), 7.59 ppm (s, 1H, C=CH—); ^{13}C NMR (deuteriochloroform): δ 11.0 (pyrazole 5-Me), 28.0 (CO_2CMe_3), 34.7 (pyrazole 1-Me), 42.6 (NCH_2), 82.7 (CO_2CMe_3), 102.9 (pyrazole C-4), 117.4 (TZD C-5), 124.0 (C=CH—), 126.0, 128.4, 130.0, 133.6 (Ph-C), 153.5 (pyrazole C-5), 162.4 (pyrazole C-3), 165.6, 167.0 (2 × C=O), 170.0 ppm (CO_2CMe_2); ms: m/z 430 $[M+H]^+$. Anal. Calcd. for $C_{21}H_{23}N_3O_5S$: C, 58.73; H, 5.40; N, 9.78. Found: C, 58.75; H, 5.42; N, 9.62.

General procedure for the preparation of pyrazole–TZDs 8a–d from 4, 5a–d, and 7. To an ice-cooled and stirred solution of **4** (1.17 g, 10 mmol) in tetrahydrofuran (30 mL) was added 60% sodium hydride (0.40 g, 10 mmol). The stirring was continued at room temperature until evolution of gas ceased. To the obtained solution was added methyl bromoacetate (**5a**) (1.53 g, 10 mmol), ethyl bromoacetate (**5b**) (1.67 g, 10 mmol), isopropyl bromoacetate (**5c**) (1.81 g, 10 mmol), or *tert*-butyl bromoacetate (**5d**) (1.95 g, 10 mmol) with stirring and ice cooling, and then the mixture was refluxed for 3 h. After removal of the deposited crystals by filtration, the filtrate was concentrated *in vacuo*. To the obtained residue was added **7** (2.16 g, 10 mmol) and sodium acetate (0.82 g, 10 mmol), and then the mixture was stirred at 140°C for 30 min. The resulting mixture was purified by column chromatography on silica gel with chloroform as the eluent to yield **8a** (2.09 g, 54%), **8b** (2.08 g, 52%), **8c** (2.50 g, 60%), and **8d** (1.76 g, 41%), respectively. The melting points and IR spectra of **8a–d** coincided with those of authentic samples prepared from **6a–d** and **7**.

General procedure for the preparation of spiro-compounds 9a–d from 8a–d and sodium hydride. To an ice-cooled and stirred solution of **8a–d** (1 mmol) in *N*,*N*-dimethylformamide (10 mL) was added 60% sodium hydride (0.08 g, 2 mmol). After the mixture was stirred at room temperature overnight, a 5% hydrochloric acid solution (20 mL) was added to the reaction mixture with stirring and ice cooling. The resulting mixture was extracted with chloroform (60 mL). The extract was dried over anhydrous sodium sulfate and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel with chloroform–acetone (4:1) as the eluent to give **9a–d**.

1′,4′,6′,7′-Tetrahydro-6′-(1,2-dihydro-1,5-dimethyl-3-oxo-2-phenyl-3H-pyrazol-4-yl)-1′-methyl-4′-[(2,4-dioxo-3-thiazolidine)acetic acid methyl ester-5-yl]spiro[thiazolidine-5,5′-[5H]indazol]-2,3′,4(2′H)-trione-3-acetic acid methyl ester (9a).. This compound was obtained as colorless needles, mp 175–177°C dec. (acetone–petroleum ether); IR (potassium bromide): ν 1755, 1733, 1702, 1682, 1649 cm^{-1} (C=O); ^1H NMR (deuteriochloroform): δ 2.18 (s, 3H, pyrazole 5-Me), 2.59–2.63 (m, 1H, 7′-H), 3.01, 3.15 (s, 6H, 2 × pyrazole 1-Me), 3.72, 3.73 (s, 6H, 2 × CO_2Me), 3.82 (dd, J = 5.2, 12.9 Hz, 1H, 6′-H), 4.30–4.32 (m, 2H, 4′- and 7′-H), 4.34, 4.42 (s, 4H, 2 × NCH_2), 4.84 (s, 1H, TZD 5-H), 7.24–7.35 (m, 6H, Ph-H), 7.39–7.46 ppm (m, 4H, Ph-H); ^{13}C NMR (deuteriochloroform): δ 11.6 (pyrazole 5-Me), 23.3 (C-7′), 35.3, 35.5 (2 × pyrazole 1-Me), 39.7 (C-6′), 42.0, 42.3 (2 × NCH_2), 44.0 (TZD C-5), 49.6 (C-4′), 52.4, 52.7 (2 × CO_2Me), 69.8 (C-5′), 101.0 (pyrazole C-4), 102.5 (C-3′a), 124.5, 125.0, 127.0, 127.2, 129.17, 129.2, 134.4, 134.6, (Ph-C), 154.5 (pyrazole C-5), 154.8 (C-7′a), 163.4 (C-3′), 164.8 (pyrazole C-3), 166.4, 167.7 (2 × CO_2Me), 168.8, 171.8 (2 × TZD C-4), 172.3,

174.6 ppm (2 × TZD C-2); ms: *m/z* 775 $[M+H]^+$. Anal. Calcd. for $C_{36}H_{34}N_6O_{10}S_2$: C, 55.80; H, 4.42; N, 10.85. Found: C, 55.57; H, 4.67; N, 10.67.

1′,4′,6′,7′-Tetrahydro-6′-(1,2-dihydro-1,5-dimethyl-3-oxo-2-phenyl-3H-pyrazol-4-yl)-1′-methyl-4′-[(2,4-dioxo-3-thiazolidine)acetic acid ethyl ester-5-yl]spiro[thiazolidine-5,5′-[5H]indazol]-2,3′,4(2′H)-trione-3-acetic acid ethyl ester (9b). This compound was obtained as colorless needles, mp 148–150°C dec. (acetone–petroleum ether); IR (potassium bromide): ν 1746, 1693 cm^{-1} (C=O); ^1H NMR (deuteriochloroform): δ 1.23–1.30 (m, 6H, 2 × CO_2CH_2Me), 2.18 (s, 3H, pyrazole 5-Me), 2.59–2.64 (m, 1H, 7′-H), 3.01, 3.14 (s, 6H, 2 × pyrazole 1-Me), 3.80–3.83 (m, 1H, 6′-H), 4.13–4.24 (m, 4H, 2 × CO_2CH_2Me), 4.32–4.41 (m, 2H, 4′- and 7′-H), 4.32, 4.40 (s, 4H, 2 × NCH_2), 4.85 (s, 1H, TZD 5-H), 7.25–7.34 (m, 6H, Ph-H), 7.38–7.50 ppm (m, 4H, Ph-H); ^{13}C NMR (deuteriochloroform): δ 11.0 (pyrazole 5-Me), 14.0, 14.1 (2 × CO_2CH_2Me), 23.3 (C-7′), 35.3, 35.5 (2 × pyrazole 1-Me), 39.7 (C-6′), 42.2, 42.5 (2 × NCH_2), 43.9 (TZD C-5), 49.7 (C-4′), 61.8, 62.1 (2 × CO_2CH_2Me), 69.8 (C-5′), 100.9 (pyrazole C-4), 102.5 (C-3′a), 124.5, 124.9, 126.1, 127.0, 129.15, 129.2, 134.3, 134.5, (Ph-C), 154.4 (C-7′a), 154.8 (pyrazole C-5), 163.3 (C-3′), 164.8 (pyrazole C-3), 165.9, 167.3 (2 × CO_2Et), 168.9, 171.9 (2 × TZD C-4), 172.4, 174.6 ppm (2 × TZD C-2); ms: *m/z* 803 $[M+H]^+$; high-resolution ms: Calcd. for $C_{38}H_{39}N_6O_{10}S_2$ 803.2169, found 803.2166. Anal. Calcd. for $C_{38}H_{38}N_6O_{10}S_2 \cdot 0.8H_2O$: C, 55.84; H, 4.88; N, 10.28. Found: C, 55.75; H, 4.92; N, 10.15.

1′,4′,6′,7′-Tetrahydro-6′-(1,2-dihydro-1,5-dimethyl-3-oxo-2-phenyl-3H-pyrazol-4-yl)-1′-methyl-4′-[(2,4-dioxo-3-thiazolidine)acetic acid isopropyl ester-5-yl]spiro[thiazolidine-5,5′-[5H]indazol]-2,3′,4(2′H)-trione-3-acetic acid isopropyl ester (9c).. This compound was obtained as colorless needles, mp 170–172°C dec. (acetone–petroleum ether); IR (potassium bromide): ν 1741, 1695 cm^{-1} (C=O); ^1H NMR (deuteriochloroform): δ 1.19–1.27 (m, 12H, 2 × CO_2CHMe_2), 2.17 (s, 3H, pyrazole 5-Me), 2.60–2.64 (m, 1H, 7′-H), 3.01, 3.14 (s, 6H, 2 × pyrazole 1-Me), 3.78–3.83 (m, 1H, 6′-H), 4.27, 4.36 (s, 4H, 2 × NCH_2), 4.31–4.32 (m, 2H, 4′- and 7′-H), 4.85 (s, 1H, TZD 5-H), 4.84–5.07 (m, 2H, 2 × CO_2CHMe_2), 7.23–7.58 ppm (m, 10H, Ph-H); ^{13}C NMR (deuteriochloroform): δ 11.6 (pyrazole 5-Me), 21.5, 21.6, 21.7, 21.8 (2 × CO_2CHMe_2), 23.4 (C-7′), 35.3, 35.6 (2 × pyrazole 1-Me), 39.8 (C-6′), 42.4, 42.8 (2 × NCH_2), 43.9 (TZD C-5), 49.7 (C-4′), 69.4, 70.2 (2 × CO_2CHMe_2), 69.8 (C-5′), 101.1 (pyrazole C-4), 102.6 (C-3′a), 124.2, 124.6, 125.0, 125.7, 126.9, 127.2, 129.1, 129.2, 134.5, 134.6, (Ph-C), 154.6 (pyrazole C-5), 154.8 (C-7′a), 163.4 (C-3′), 164.7 (pyrazole C-3), 165.4, 166.7 (2 × CO_2Pr^i), 168.8, 171.2 (2 × TZD C-4), 172.4, 174.6 ppm (2 × TZD C-2); ms: *m/z* 831 $[M+H]^+$. Anal. Calcd. for $C_{40}H_{42}N_6O_{10}S_2$: C, 57.82; H, 5.09; N, 10.11. Found: C, 57.42; H, 5.16; N, 10.13.

1′,4′,6′,7′-Tetrahydro-6′-(1,2-dihydro-1,5-dimethyl-3-oxo-2-phenyl-3H-pyrazol-4-yl)-1′-methyl-4′-[(2,4-dioxo-3-thiazolidine)acetic acid *tert*-butyl ester-5-yl]spiro[thiazolidine-5,5′-[5H]indazol]-2,3′,4(2′H)-trione-3-acetic acid *tert*-butyl ester (9d).. This compound was obtained as colorless needles, mp 155–157°C dec. (acetone–petroleum ether); IR (potassium bromide): ν 1742, 1694 cm^{-1} (C=O); ^1H NMR (deuteriochloroform): δ 1.27–1.47 (m, 18H, 2 × CO_2CMe_3), 2.20 (s, 3H, pyrazole 5-Me), 2.87–2.95 (m, 1H, 7′-H), 3.03, 3.20 (s, 6H, 2 × pyrazole 1-Me), 3.79–3.84 (m, 1H, 6′-H), 4.21, 4.26 (s, 4H, 2 × NCH_2), 4.23–4.25 (m, 2H, 4′- and 7′-H), 4.85 (s, 1H, TZD 5-H), 7.24–7.28 (m, 3H, Ph-H), 7.32–7.48 ppm (m, 7H, Ph-H); ^{13}C NMR (deuteriochloroform): δ 11.6 (pyrazole 5-Me), 23.7 (C-7′), 27.9, 27.95, 27.99, 28.0 (2 × CO_2CMe_3), 35.0, 35.6 (2 × pyrazole 1-Me), 39.6 (C-6′), 42.9, 43.2 (2 × NCH_2), 43.9 (TZD C-5), 49.8 (C-4′), 69.8 (C-5′), 82.4, 83.1 (2 × CO_2CMe_3), 101.3 (pyrazole C-4), 102.5 (C-3′a), 124.5, 124.9, 127.0, 127.2, 129.18, 129.2, 134.5, (Ph-C), 154.5 (pyrazole C-5 and C-7′a), 163.3 (C-3′), 164.6 (pyrazole C-3), 165.1, 166.3 (2 × CO_2Bu^t), 168.7, 171.8 (2 × TZD C-4), 172.3, 174.6 ppm (2 × TZD C-2); ms: *m/z* 859 $[M+H]^+$. Anal. Calcd. for $C_{42}H_{46}N_6O_{10}S_2$: C, 58.73; H, 5.40; N, 9.78. Found: C, 59.01; H, 5.51; N, 9.81.

Tumor cell culture and MTT assay.. A549 and HeLa cells were obtained from DS Pharma Biomedical (Osaka, Japan). B16F10 cells were purchased from American Type Culture Collection (Manassas, VA). Tumor cells were cultured in Roswell Park Memorial Institute 1640 medium or Dulbecco's Modified Eagle Medium (DMEM) at 37°C with 5% carbon dioxide and 95% air, supplemented with fetal bovine serum and/or penicillin, streptomycin, or kanamycin. The cells were seeded onto 96-well plates. The inhibition of the cellular growth was estimated using MTT assay according to Mosmann [41] and our previous methods [42].

3T3-L1 cell culture and differentiation assay. 3T3-L1 preadipocytes, which were obtained from DS Pharma Biomedical (Japan), were cultured in DMEM and differentiated by a modified previously described protocol [43]. After 2 days, postconfluent preadipocytes were treated for 2 days with complete medium containing dexamethasone (1 μM), insulin (0.17 μM), and 3-mehtyl-1-isobutylxanthine (0.5 mM). After medium replacement, they were incubated for further 2 days with postdifferentiation medium and for the last 2 days with only complete medium. The medium was changed every 2 days. Cells were treated the whole time period with either dimethyl sulfoxide-d_6 (DMSO) as a negative control, pioglitazone as a positive control, or the

synthesized compounds until day 12 of differentiation, after which the cells were washed with phosphate-buffered saline and stained with Oil Red O. For quantification, the dye was extracted with isopropanol and the absorption was measured at 540 nm.

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