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Synthesis of Polymeric Thiazolidinediones and L-Ascorbic Acid Towards the Development of Insulin-Sensitizer

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Polymers, containing 5-(4-*O*-methylacryloylbenzyl)thiazolidine-2,4-dione [MABTZD]; poly (MABTZD), poly(MABTZD-*co*-AA) and poly(MABTZD-*co*-AMAA), were prepared, and identified by FT-IR, ^{1}H- and ^{13}C-NMR spectra. The MABTZD unit contents in poly(MABTZD-*co*-AA) and poly(MABZD-*co*-AMAA) were 11.3 and 27.7 mol %, respectively. The number average molecular weights of the polymers, as determined by GPC, ranged from 16,800 to 22,300, and with polydispersity indices of 1.2~1.4.

Key words: Thiazolidinedione, Polymer, L-Ascorbic acid, Diabetes

INTRODUCTION

Type 2 diabetes, also known as non-insulin dependent diabetes mellitus (NIDDM), is the most prevalent endocrine disease in the world, which is characterized by insulin resistance in the liver and peripheral tissues. Recently, a new class of insulin-sensitizing drugs, called thiazolidinedione (TZD), such as Avandia (rosiglitazone) and Actos (pioglitazone), has been found to provide improvements in the blood glucose in type 2 diabetics due to an insulin sensitizing mechanism (Fujiwara, *et al.*, 1988; Cantello, *et al.*, 1994; Meguro, *et al.*, 1985). The molecular mechanism of the antidiabetic TZDs involves activation of peroxisome proliferator-activator receptor gamma (PPARγ), a member of a family of ligand activated nuclear hormone receptors (Lehman, *et al.*, 1995). It has been well published that the critical pharmacophore of antidiabetic TZDs is the hydrophilic thiazolidinedione moiety. In addition, L-ascorbic acid was found to be a major contributor to the serum total antioxidant activity (Frei *et al.*, 1991). Both NIDDM patients (Maxwell *et al.*, 1997) and diabetic rats (Torres *et al.*, 1999) had significantly lower total antioxidant activities compared to the controls. This prompted us to synthesize novel polymeric TZDs, which could release the small molecule from the polymer and also add other features by the hanging of a 2nd molecule. Herein, the syntheses of a homopolymer, with hydrophilic thiazolidinedione alone, and copolymers, with TZD and acrylic acid or ascorbic acid, are reported.

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MATERIALS AND METHODS

Unless indicated, the reagents and solvents were purchased from Aldrich chemicals, and used without purification, with the following exceptions; ethyl ether and tetrahydrofuran were distilled from sodium benzophenone ketyl. Methanol, toluene, pyridine, and dimethyl formamide were distilled from calcium hydride under nitrogen. Flash column chromatography was performed using silica gel 60 (230-400 mesh, Merck) with the indicated solvents. Thin-layer chromatography (TLC) was performed using Kieselgel 60 F_{254} plates (Merck). Infrared spectra were recorded on a Jasco FT/IR 430 spectrometer. ^{1}H- and ^{13}C-NMR spectra were recorded on Varian YH 400 spectrometer, as solutions in deuteriochloroform ($CDCl_3$) or deuteriodimethyl sulfoxide ($(CD_3)_2SO$). Chemical shifts are expressed in parts per million (ppm, d) downfield from tetramethylsilane, which was used as an internal standard. ^{1}H-NMR data are reported in the order of chemical shift, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet and/or multiple resonance), number of protons and coupling constant in hertz (Hz).

5-(4-Hydroxybenzylidene)thiazolidine-2,4-dione (2)

A mixture of 4-hydroxybenzaldehyde (5.18 g, 41.57 mmol), 2, 4-thiazolidinedione (4.87 g, 41.57 mmol), benzoic acid (6.09 g, 49.88 mmol) and anhydrous toluene (50 mL)

was stirred at 130°C for 5 h. The reaction mixture was chilled and filtered. The filtercake was washed with 50% methanol solution to afford 7.88 g (86%) of the title compound as a yellow powder: R_f = 0.35 (*n*-hexane/EtOAc = 2:1); IR (neat) 3405, 3125, 3003, 2792 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 12.41 (br s, 1H), 10.27 (br s, 1H), 7.66 (s, 1H), 7.42 (d, 2H, *J* = 8.4 Hz), 6.87 (d, 2H, *J* = 8.4 Hz); ^{13}C-NMR (100 MHz, DMSO-d_6) δ 168.1, 167.6, 159.9, 132.4, 132.3, 123.9, 119.0, 116.3.

5-(4-Hydroxybenzyl)thiazolidine-2,4-dione (3)

To a mixture of **2** (1.65 g, 7.04 mmol), dimethyl formamide (20 mL) and methanol (30 mL) was added 10% Pd/C (1.65 g, 15.51 mmol), and the resulting mixture was vigorously shaken in 30 psi of hydrogen for 7 days. The reaction mixture was filtered, and the filtrate was concentrated under reduced pressure to afford the title compound as a yellow powder: ^{1}H-NMR (400 MHz, DMSO-d_6) δ 11.95 (br s, 1H), 9.31 (br s, 1H), 6.99 (d, 2H, *J* = 8.4 Hz), 6.65 (d, 2H, *J* = 8.4 Hz), 4.78 (dd, 1H, *J* = 9.2, 4.0 Hz), 3.21 (dd, 1H, *J* = 14.0, 4.0 Hz), 2.95 (dd, 1H, *J* = 14.0, 9.2 Hz); ^{13}C-NMR (100 MHz, DMSO-d_6) δ 175.8, 171.8, 156.4, 130.3, 126.8, 115.2, 53.3, 36.4.

5-(4-*O*-Methylacryloylbenzyl)thiazolidine-2,4-dione [MABTZD] (4)

To a mixture of **3** (1.50 g, 6.73 mmol) and anhydrous ethyl acetate (15 mL) was added methacrylic anhydride (1.20 mL, 8.07 mmol) and pyridine (1.09 mL, 13.45 mmol). The mixture was stirred at room temperature for 12 h. The reaction solution was washed with water, dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (eluant, *n*-hexane/ethyl acetate = 1:1 v/v) to afford 1.16 g (60%) of the title compound as a white powder: R_f = 0.5 (*n*-hexane/EtOAc = 1:1); IR (neat) 3407, 2256, 2129, 1695, 1509, 1320, 1205, 1169, 1133, 1025, 825, 764 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 12.08 (br, 1H), 7.29 (d, 2H, *J* = 8.4 Hz), 7.11 (d, 2H, *J* = 8.4 Hz), 6.23 (s, 1H), 5.85 (d, 1H, *J* = 1.6 Hz), 4.90 (dd, 1H, *J* = 3.8, 9.2 Hz), 3.39 (dd, 1H, *J* = 4.4, 14.0 Hz), 3.11 (dd, 1H, *J* = 9.2, 14.0 Hz), 1.98 (s, 3H); ^{13}C-NMR (100 MHz, DMSO-d_6) δ 175.6, 171.6, 165.2, 149.6, 135.3, 134.4, 130.4, 127.7, 121.7, 52.6, 36.8, 18.0.

3-*O*-Allyl-2-*O*-methyl-L-ascorbic acid [AMAA] (6)

To a stirred solution of triphenyl phosphine (1.36 g, 5.19 mmol) in dry THF (20 mL), diethyl azodicarboxylate (0.8 mL, 5.08 mmol) was added dropwise at -78°C. After 15 min, a white solid (Mitsunobu betaine) formed. The stirring was continued at the same temperature for an additional 10 min. A solution of L-ascorbic acid (706 mg, 4.01 mmol) in DMF (20 mL) was then added through a cannula at -78 °C. The cooling bath was removed, and after all of the solid had dissolved, allyl alcohol (0.4 mL, 5.88 mmol) was added. The mixture was allowed to warm to 25°C and stirred for 24 h. The solvent was removed under reduced pressure. The residue was purified by flash column chromatography (eluant, methylene chloride/methanol = 10:1) to afford 679.4 mg (79%) of the title compound as a white powder: R_f = 0.33 (methylene chloride/methanol = 10:1); ^{1}H-NMR (400 MHz, DMSO-d_6) δ 8.81 (s, 1H), 6.00 (m, 1H), 5.40 (dq, 1H, *J* = 12.3, 1.6 Hz), 5.25 (dq, 1H, *J* = 10.4, 1.6 Hz), 4.98 (d, 1H, *J* = 6.4 Hz), 4.91 (ddt, 2H, *J* = 13.2, 5.2, 1.6 Hz), 4.85 (ddt, 2H, *J* = 13.2, 5.2, 1.6 Hz), 4.78 (d, 1H, *J* = 1.2 Hz), 3.65 (m, 1H), 3.35 (s, 2H); ^{13}C-NMR (100 MHz, DMSO-d_6) δ 170.0, 149.7, 132.8, 118.9, 117.4, 74.0, 70.4, 68.1, 61.3.

Poly-[5-(4-*O*-methylacryloylbenzylidene)thiazolidine-2,4-dione] [Poly(MABTZD)] (7)

A solution of MABTZD (100 mg, 0.35 mmol) and BPO (4 mg, 0.02 mmol) as an initiator in dry THF (4 mL) was introduced into a Pyrex tube. The tube was sealed after flushing twice with a stream of N_2 and placed in a regulated thermostat bath at 70°C for 48 h. The solution obtained was slowly dropped into 20 mL of *n*-hexane to precipitate the polymer. The precipitated polymer was collected by filtration and washed several times with chloroform, and the obtained homopolymer was dried to a constant weight at room temperature under vacuum. The conversion was 61%; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 12.07, 7.29, 7.01, 4.88, 3.11, 2.35~1.00, 1.29; ^{13}C-NMR (100 MHz, DMSO-d_6) δ 175.2, 171.0, 167.1, 150.3, 136.9, 128.3, 121.3, 57.0, 42.6, 36.2, 32.2, 21.9.

Poly-[5-(4-*O*-methylacryloylbenzyl)thiazolidine-2,4-dione-co-acrylic acid] [Poly(MABTZD-*co*-AA)] (8)

A solution of MABTZD (150 mg, 0.53 mmol) and acrylic acid (AA; 0.04 mL, 0.53 mmol) with BPO (7.52 mg, 0.03 mmol) as an initiator in dry THF (4 mL) was introduced into a Pyrex tube. The tube was sealed after flushing twice with a stream of N_2. The preparation procedure for poly(MABTZD-*co*-AA) was the same as that described for the homopolymerization of MABTZD with the exception of the monomer pairs. The copolymerization conversion was 79%; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 12.24, 12.03, 7.20, 7.01, 4.87, 3.11, 1.42~1.20, 1.71; ^{13}C-NMR (100 MHz, DMSO-d_6) δ 179.2, 175.2, 173.0, 167.1, 150.3, 137.0, 128.3, 121.4, 57.0, 42.6, 37.5, 35.3, 28.8, 19.8, 17.2.

Poly-[5-(4-*O*-methylacryloylbenzyl)thiazolidine-2,4-dione-*co*-3-*O*-allyl-2-*O*-methyl-L-ascorbic acid] [Poly(MABTZD-*co*-AMAA)] (9)

The thermal polymerization of MABTZD and AMAA was performed as described for the copolymerization of

MABTZD and AA with the exception of the monomer pairs. The conversion was 87%; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 12.07, 7.28, 7.04, 5.06, 4.26, 3.60, 3.38, 3.13, 2.09~1.30, 1.76; ^{13}C-NMR (100 MHz, DMSO-d_6) δ 175.2, 174.7, 171.1, 149.0, 134.0, 131.7, 129.8, 121.0, 118.3, 106.5, 86.8, 77.5, 73.0, 66.6, 52.1, 38.4, 36.0, 24.7.

Measurement of average molecular weight

The average molecular weights and polydispersity (PDI = Mw/Mn) were determined by gel permeation chromatography (GPC) using Water GPC 410 with a refractive index detector and four μ-Styragel columns with pore sizes of 105, 104, 103, and 500 Å connected in series. The standard and eluant used were polystyrene and DMF, respectively, at a flow rate of 1 mL/min (40°C).

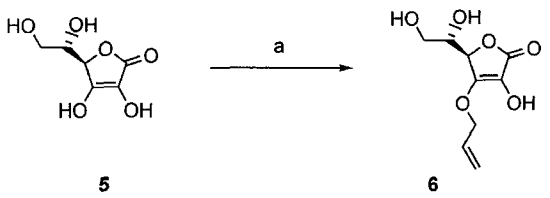
PPAR Transactivation assay

CV-1 cells were seeded at 2×10^4 cells/well and cultured for 24 h at 37°C. The cells were cotransfected with pUAS, pRL-TK and pCMX-GalPx for 3 h at 37°C. The transfected cells were treated with test compounds for 24 h with DMSO (0.1 %) was used as a blank and GW409544 as a positive control. The luciferase activity was determined as the 'fold activation' relative to the positive control.

RESULTS AND DISCUSSION

Chemistry

The monomer, 5-(4-*O*-methylacryloylbenzyl)thiazolidine-2,4-dione (MABTZD), was synthesized by the reaction of methacrylic anhydride with compound **3**, which was prepared by condensation of 4-hydroxybenzaldehyde with thiazolidinedione, followed by hydrogenation of the compound **2** (Scheme 1). Monomer **6** was prepared by Mitsunobu reaction of ascorbic acid with allyl alcohol (Scheme 2). The homopolymer and copolymers of MABTZD, with acrylic acid (AA) and 3-*O*-allyl-2-*O*-methyl-L-ascorbic acid (AMAA), respectively, were synthesized by thermal polymerizations in the presence of benzoyl peroxide as an initiator (Scheme 3).



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Scheme 2. Preparation of 3-*O*-allyl-2-*O*-methyl-L-ascorbic acid [AMAA]. Reagents and conditions: (a) allyl alcohol, DEAD, Ph_3P, THF/DMF, rt, 24 h, 79%.

Solubilities of MABTZD and its polymers

The solubilities of MABTZD and the polymers were examined and are listed in Table I. The prepared samples (5 mg) were poured in the solvents (1 mL) at room temperature. The mixtures were shaken until the samples dissolved. The MABTZD and its polymers were soluble in methanol, THF, DMSO and DMF. In chloroform, the MABTZD showed good solubility, but the synthesized polymers were insoluble due to their high molecular weights. All of the prepared compounds were insoluble in H_2O and *n*-hexane.

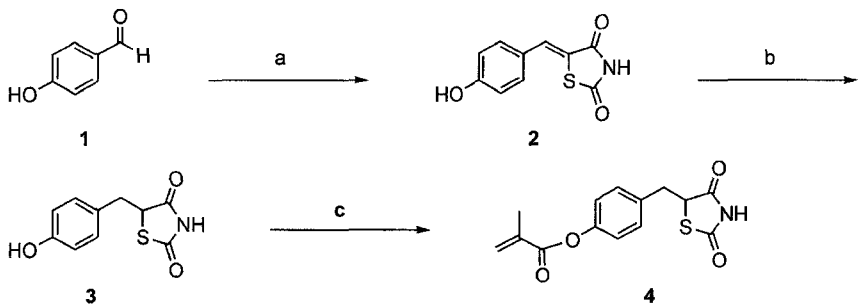
Average molecular weight of polymers

The average molecular weights and polydispersity indices of the polymers were determined by gel permeation chromatography (GPC), and are listed in Table II.

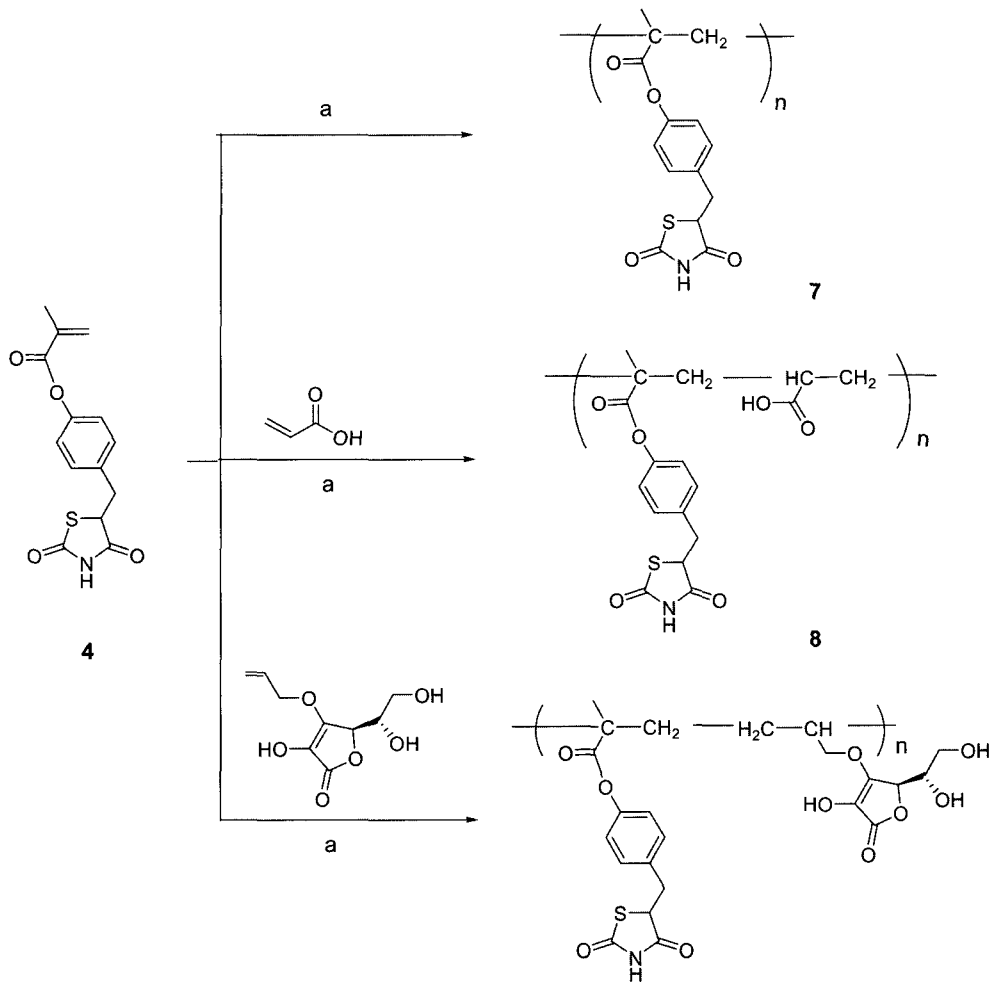
Contents of MABTZD moiety in copolymers

The MABTZD contents in the synthesized copolymers, as calculated from the C, N, and H data obtained by elemental analysis, are listed in Table III. The MABTZD contents in poly(MABTZD-*co*-AA) and poly(MABTZD-*co*-AMAA) were 11.3 and 27.7 mol %, respectively.

The polymers **7**-**9** were evaluated for their abilities to activate PPARγ in a transactivation assay, but no activity was shown. The PPARγ activity of monomer **4** was also



Scheme 1. Preparation of 5-(4-*O*-methylacryloylbenzyl)thiazolidine-2,4-dione [MABTZD]. Reagents and conditions: (a) thiazolidine-2,4-dione, piperidine, benzoic acid, toluene, 130, 5 h, 86%; (b) H_2, 10% Pd/C, MeOH, DMF, rt, 7 d, 73%; (c) methacrylic anhydride, pyridine, ethyl acetate, rt, 12 h, 60%.



Scheme 3. Preparation of polymers containing 5-(4-*O*-methylacryloylbenzyl)thiazolidine-2, 4-dione. Reagents and conditions: (a) benzoyl peroxide, THF, 70°C, 48 h.

Table I. The solubilities of MABTZD and Its polymers

Solvent	MABTZD	Poly (MABTZD)	Poly(MABTZD-*co*-AA)	Poly(MABTZD-*co*-AMAA)
H_2O	IS[a]	IS	IS	IS
Methanol	S[b]	S	S	S
THF	S	S	S	S
DMF	S	S	S	S
DMSO	S	S	S	S
Chloroform	S	IS	IS	IS
n-hexane	IS	IS	IS	IS

[a]IS: Insoluble, [b]S: Soluble

evaluated, as it might be conceivable that the lack of PPARγ agonism by the polymers was due to the problems of releasing the monomers from the polymer. As expected, the TZD monomer, which consists of one segment of the whole pharmacophore, also showed no PPARγ activity.

Table II. Average molecular weights and polydispersity of the polymers

Polymers	Mn[a]	Mw[a]	Mw/Mn[a]
Poly(MABTZD)	16,800	19,700	1.2
Poly(MABTZD-*co*-AA)	21,400	29,600	1.4
Poly(MABTZD-*co*-AMAA)	22,300	31,500	1.4

[a]The number (*Mn*) and weight (*Mw*) average molecular weights of the polymers were determined by GPC in DMF.

Table III. Elemental compositions and MABTZD contents in the copolymers

Compound	E.A. (%)				MABTZD contents in copolymer (mole%)[a]
	C	H	N	S	
Poly(MABTZD-*co*-AA)	53.85	5.27	2.83	3.75	11.33
Poly(MABTZD-*co*-AMAA)	54.53	5.36	2.65	3.75	27.73

[a]The compositions of copolymers were determined by elemental analysis.

In summary, studies on a polymeric antidiabetic, and the first synthesized polymeric thiazolidinediones, which are composed with only hydrophilic thiazolidinedione, have been undertaken, as thiazolidinedione is conceivably labile under many chemical conditions. The thiazolidinedione homopolymer, poly(MABTZD), and the copolymers, poly(MABTZD-*co*-AA) and poly(MABTZD-*co*-AMAA), were prepared by thermal polymerization. All the prepared polymers were evaluated for their abilities to activate PPARγ in a transactivation assay. However these partially constructed polymeric TZDs showed no PPARγ agonism. The synthesis of a polymer, employing the synthetic route optimized in this study, with the entire structural features of thiazolidinedione antidiabetics is underway.

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REFERENCES

Fujiwara, T., Yoshioka, S., Yoshioka, T., Ushiyama, I., and Horikoshi, H., Characterization of new oral antidiabetic agent CS-045. Studies in KK and ob/ob mice and Zucker fatty rats. *Diabetes,* 37, 1549-1558 (1988).

Cantello, B. C. C., Cawthorne, M. A., Cottam, G. P., Duff, P. T., Haigh, D., Hindley, R. M., Lister, C. A., Smith, S. A., and Thurlby, P. L., [[omega-(Heterocyclylamino)alkoxy]benzyl]-2,4-thiazolidinediones as potent antihyperglycemic agents. *J. Med. Chem.,* 37, 3977-3985 (1994).

Meguro, K. and Fujita, T., Thiazolidinedione derivatives, and their medicinal compositions. EP 0155845 (1985).

Lehman, J. M., Moore, L. B., Smith- Oliver, T. A., Wilkison, W. O., Willson T. M., and Kliewer, S. A., An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor gamma (PPAR gamma). *J. Biol. Chem.,* 270, 12953-12956 (1995).

Frei, B., Stocker, R., England, L., and Ames, E. N., Plenum Press, New Work, pp 155-164 (1991).

Maxwell, S. R., Thomason, H., Sandler, D., Leguen, C., Baxter, M. A., Thorpe, G. H., Jones, A. F., and Barnett, A. H. Antioxidant status in patients with uncomplicated insulin-dependent and non-insulin-dependent diabetes mellitus., *Eur. J. Clin. Inest.,* 27, 484-490 (1997).

Torres, M. D., Canal, J. R., and Perez, C., Oxidative stress in normal and diabetic rats. *Physiol. Res.* 48, 203-208 (1999).