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Synthesis and Structure–Activity Relationship Studies of Cinnamic Acid-based Novel Thiazolidinedione Antihyperglycemic Agents

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Abstract—A number of 2,4-thiazolidinedione derivatives of -phenyl substituted cinnamic acid were synthesized and studied for their PPAR agonist activity. The *E*-isomer of cinnamic acid, **11**, showed moderate PPAR transactivation. The corresponding *Z*-isomer, **23**, and double bond reduced derivative, **15**, were found to be much less potent. Although the *E*-isomer showed a moderate PPARγ transactivation, it demonstrated a strong glucose-lowering effect in a genetic rodent model of diabetes. Results of pharmacokinetic, metabolism and permeability studies are consistent with **11** being an active prodrug with an active metabolite, **14**, that has similar glucose lowering and PPARγ agonist properties.

Introduction

Type 2 diabetes (DM2) is having an increasingly adverse impact on morbidity, mortality and overall health care costs in North American and Western European countries.[1,2] The incidence of the disease, currently underdiagnosed, is expected to reach 215 million by the year 2010.[3] Early stages of the disease are characterized by tissue resistance to the effects of insulin secreted by pancreatic beta cells. As the disease progresses, the pancreas' ability to continue increased production of insulin diminishes over time. As insulin production declines in the face of insulin resistance, glucose disposal from the circulation into muscle is diminished and suppression of hepatic glucose output is decreased. DM2 is the result of these events and is manifested clinically by hyperglycemia. The increased and sustained blood glucose levels eventually give rise to retinopathy, neuropathy, nephropathy.[4] The macrovascular (atherosclerotic) complications of DM2 are less closely linked to hyperglycemia but nonetheless contribute to substantial morbidity in DM2. The large vessel complications directly result from the altered metabolic milieu in DM2.

When recommended dietary modification and exercise fail to control elevated blood glucose levels, pharmacological therapy[5] is required to restore normoglycemia, reduce the attendant sequelae and delay the need for insulin therapy. Pharmacological agents, available or under investigation, include drugs that improve insulin resistance or increase insulin secretition. Metformin, a biguanide, acts primarily by decreasing hepatic glucose output and increasing peripheral glucose utilization.[6] It is a first line therapeutic option for DM2. Sulfonylureas stimulate insulin secretion by blocking ATP-dependent potassium channels[7] but are associated with a significant risk of hypoglycemia.[8] The meglitinides are a different class of drug but are similar in action to sulfonylureas. Recently introduced thiazolidinediones (TZDs)[9] act by improving peripheral insulin sensitivity. These agents act as agonists of the peroxisome proliferator-activated receptor gamma (PPARγ)[10] but their exact mechanism of action in reducing insulin resistance is unclear.[11] During the last decade, an exhaustive search for novel agents based on the thiazolidinedione ciglitazone[12] has yielded several development candidates, as

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well as the marketed drugs pioglitazone, **1**,[13] rosiglitazone, **2**,[14] and troglitazone (recently withdrawn from the market), **3**.

We have previously shown that the α-phenyl substituted cinnamic acid derivative, **4**, possessed a weak antihyperglycemic effect, possibly by an interaction with insulin receptor[15] in various animal models.[16] We postulated that linking this molecule with a 2,4-thiazolidinedione (TZD) moiety could provide compounds retaining the original glucose-lowering activity and, additionally, an affinity for PPAR, and that such compounds would be useful for treating Type 2 diabetes.

This paper reports synthesis and biological activities of 2,4-thiazolidinedione analogues of **4** and related cinnamic and phenylpropionic acid and esters. Compound **11** has shown moderate in vitro PPARγ transactivation and strong glucose-lowering activity in animal model of Type 2 diabetes (Chart 1).

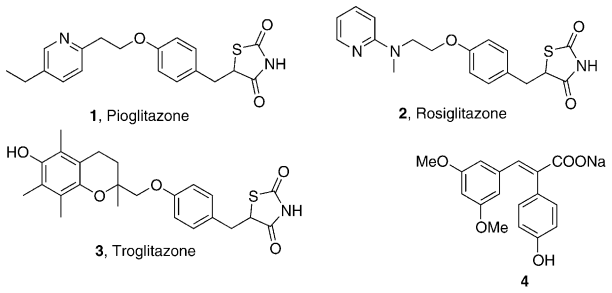


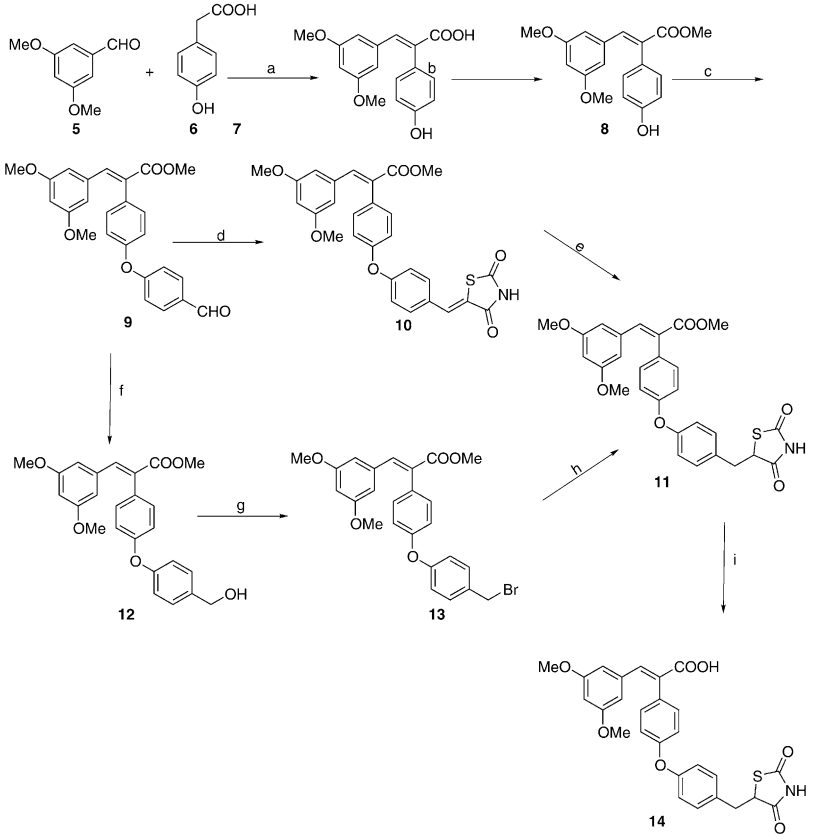
Chart 1.

Results and Discussion

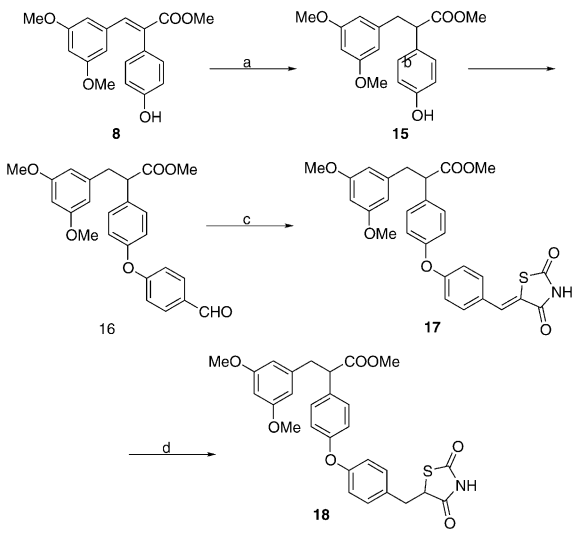
Chemistry

The procedure used for synthesis of thiazolidinediones is shown in Scheme 1. Perkin condensation of 3,5-dimethoxybenzaldehyde **5** with 4-hydroxyphenylacetic acid **6** yielded the α-phenyl substituted cinnamic acid **7** exclusively as the *E*-isomer. The geometry of the double bond was confirmed by ^{1}H NMR comparison with the reported compound.[17] Esterification of **7** followed by condensation with 4-fluorobenzaldehyde yielded **9**. Knovenagel condensation of aldehyde **9** with 2,4-thiazolidinedione in the presence of piperidinium benzoate with azeotropic removal of water gave a good yield of **10**.

A major challenge was selective hydrogenation of one of the double bonds in order to produce compounds **11**, **17** and **18** (Scheme 2). Reduction of **10** with magnesium/methanol was nonselective and yielded a mixture of products. Zinc-acetic acid reduction gave a mixture of polar products. Hydrogenation with 10% palladium on



Scheme 1. Reagents and conditions: (a) acetic anhydride, Et_3N, 6 h, 130 °C, 47%; (b) MeOH, H_2SO_4, 20 h, reflux, 97%; (c) 4-fluorobenzaldehyde, NaH, DMF, 18 h, 80 °C, 77%; (d) 2,4-thiazolidinedione, piperidine, benzoic acid, toluene, 5 h, reflux, 86%; (e) Pt/C(10%), AcOH–$HCOONH_4$, 15 h, 125 °C, 64%; (f) $NaBH_4$, EtOH, 1 h, 25 °C, quantitative; (g) PBr_3, CH_2Cl_2, 25 °C, 1 h, 99%; (h) BuLi, 2,4-thiazolidinedione, THF, 0 °C, 45 min, 15%; (i) aqueous NaOH, MeOH, 15 h, 25 °C, 73%.



Scheme 2. Reagents and conditions: (a) Pd/C (10%), H_2, 18 h, 25 °C, quantitative; (b) 4-fluorobenzaldehyde, NaH, DMF, 18 h, 80 °C, 69%; (c) 2,4-thiazolidinedione, piperidine, benzoic acid, toluene, 2 h, reflux, 81%; (d) Pd/C (10%), H_2 (60 psi), 48 h, 25 °C, 38%.

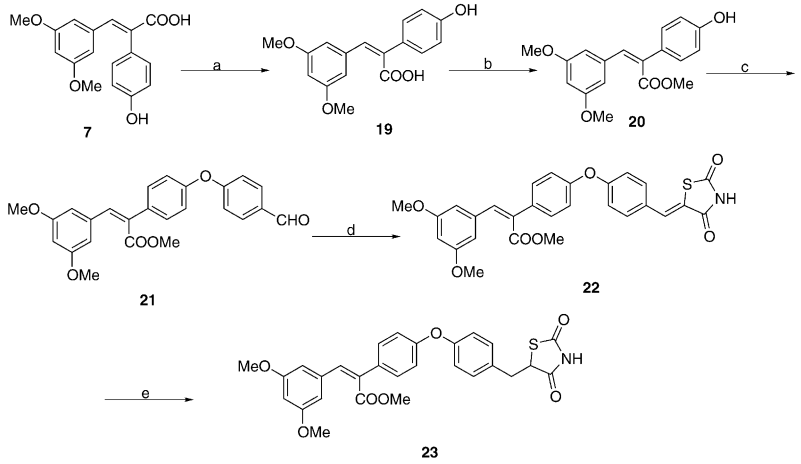
carbon as catalyst in 1,4-dioxane yielded a mixture of **11** and **18** in a ratio of 6:4. Separation of the compounds from this mixture was only possible by reverse-phase chromatography on C-18 silica. These problems were overcome in several ways. Hydrogenation of **10** using ammonium formate as hydrogen donor in the presence of palladium catalyst[18] produced minimal amounts of the over-reduced product **18**, and isolation of **11** in high purity was possible by repeated crystallization from methanol. Changing the catalyst to 10% platinum on carbon further reduced byproduct formation and increased the isolated yield of **11** from 50 to 64%. In another attempt to make **11**, the aldehyde **9** was reduced to alcohol **12** which upon treatment with PBr_3 yielded the bromo compound **13** in high yield. The bromo compound was condensed with 2,4-thiazolidinedione anion generated by BuLi to produce **11** in low yield.

It was difficult to synthesize **18** in good yield from either **10** or **11** by palladium catalyzed hydrogenation due to poisoning of the catalyst by the 2,4-thiazolidinedione moiety in the molecule; the resulting mixtures contained **18** as a minor product. To solve this problem (as shown in Scheme 2), **8** was first reduced, by using 10% palladium on carbon as catalyst, to **15** quantitatively followed by coupling with 4-fluorobenzaldehyde and 2,4-thiazolidinedione to furnish **17** in good yield. Reduction of **17** with palladium on carbon catalyst for a longer period of time and catalyst renewal halfway through the reaction followed by chromatographic purification over C-18 reverse-phase silica gel produced **18** in moderate yield.

The synthetic strategy adopted to prepare **23**, the corresponding *Z*-isomer of **11**, is outlined in Scheme 3. Prolonged heating of **7** with acetic anhydride and triethylamine[19] yielded the corresponding *Z*-isomer **19** in 13% yield. Compound **19** was converted to the corresponding methyl ester **20** by acid catalyzed esterification. Coupling of **20** with 4-fluorobenzaldehyde in presence of sodium hydride resulted in compound **21**. No significant racemization was observed in afore mentioned two steps. Interestingly, the reaction of 2,4-thiazolidinedione with **21**, in order to produce **22**, showed minimal isomerization of the cinnamic acid double bond and resulted in a mixture of *E*- and *Z*-isomers in a ratio of 1:7, respectively. Reduction was carried out without further purification and the final product was purified by preparative HPLC to yield **23**.

Pharmacological evaluation

Antidiabetic compounds of the TZD class increase peripheral tissue sensitivity to insulin via PPARγ activation. Compound **4** was tested for PPARγ agonist activity in a reporter gene assay at five different concentrations (0.063, 0.25, 1.0, 4.0, and 16 μM) and did not show any effect. Analogues of **4** were made by introducing the TZD moiety into the molecule, with the goal of introducing PPARγ agonist activity. The three possible methyl ester analogues with double bond(s) at different positions (**10**, **11**, and **17**), the methyl ester without any double bond **18**, the free acid analogue **14** of compound **11**, and the *Z*-isomer **23** of **11** were made and tested.



Scheme 3. Reagents and conditions: (a) acetic anhydride, Et_3N, 24 h, 125 °C, 13%; (b) MeOH, H_2SO_4, 18 h, reflux, 35%; (c) 4-fluorobenzaldehyde, NaH, DMF, 18 h, 80 °C, 74%; (d) 2,4-thiazolidinedione, piperidine, benzoic acid, toluene, 5 h, reflux, 91%; (e) Pd/C (10%), ammonium formate, acetic acid, 20 h, 120 °C, 15%.

Agonist activity on PPARγ was explored in an in vitro system using HEK293 cells transfected with a human PPARγ2 expression vector and a PPRE-luciferase reporter gene (PPAR responsive element-minimal promoter-firefly luciferase chimeric cDNA). Cells were cotransfected with renilla luciferase to control for transfection efficiency. Compounds were added to the transfected cells and over a dilution series of at least seven concentrations. Cells were cultured for 24 h, then lysed and activation determined using a luciferase assay system (Promega, WI) and read in a luminometer. The results from this in vitro transactivation assay for the tested compounds are summarized in Table 1. The most potent compound in this series was found to be **11** (EC_{50} of 0.28 μM) which had approximately one-thirtieth the activity of rosiglitazone, **2** (EC_{50} of 0.009 μM) in the same assay. The maximal activity of compound **11** was equivalent in magnitude to rosiglitazone. The potency for compounds **10**, containing two double bonds and **14**, the corresponding acid analogue of **11** were less than that of compound **11**. Both the compounds **17** and **18** were devoid of the cinnamic acid double bond and essentially inactive. The *Z*-isomer **23** showed less than one-tenth the potency of compound **11** in this assay. This indicates that the geometry of the double bond is critical to activity.

Table 1. Induction of PPARγ mediated luciferase activity by thiazolidinediones[a]

Compd	EC_{50} (μM + SD)
1 (rosiglitazone)	0.009 ± 0.007
10	1.136
11	0.284 ± 0.036 ($n = 5$)[b]
14	0.690 ± 0.038 ($n = 2$)
17	23.9
18	57.7
23	3.686 ± 1.454 ($n = 2$)

[a]Results of several independent experiments. Each experiment contains at least seven different concentrations of drug treatment (between 0.1 to 30 μM) and each concentration was tested in triplicate. EC_{50} values were calculated by non-linear regression analysis using GraphPad Prism (San Diego, CA, USA) software.
[b]*n*, number of independent experiments.

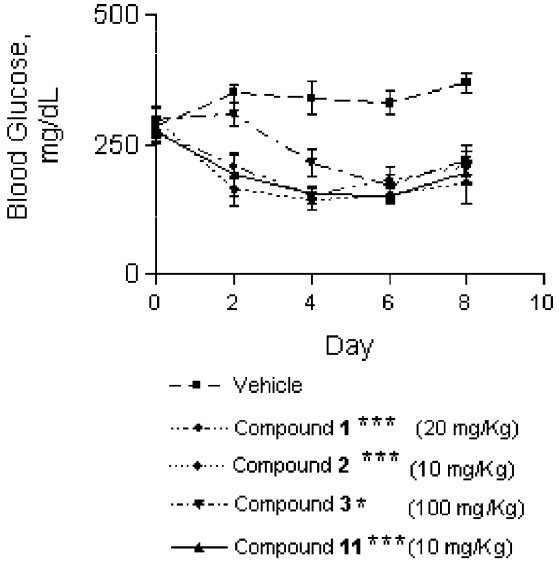


Figure 1. Blood glucose: group mean blood glucose levels in *ob*/*ob* mice administered vehicle, **11**, or comparators pioglitazone, **1**, rosiglitazone, **2** and troglitazone, **3**. ***$p < 0.001$, *$p < 0.05$.

Based on these in vitro results, compound **11** was evaluated in the *ob*/*ob* mouse model of Type 2 diabetes for its oral antihyperglyemic activity. The effects of **11** at a dose of 10 mg/kg body weight were compared to vehicle, as well as to comparators of the same class, pioglitazone, **1** (20 mg/kg), rosiglitazone, **2** (10 mg/kg), and troglitazone, **3** (100 mg/kg), used at dose levels known to be maximally effective. Compounds were administered by oral gavage for nine days to groups of five young male *ob*/*ob* diabetic mice (Fig. 1). Compound **11** at 10 mg/kg rapidly lowered blood glucose from 284 ± 57 mg/dL to a low of 151 ± 43 mg/dL on Day 6, and maintained stable levels in the normal range throughout the study. Compounds **1**, **2**, **3**, and **11** showed statistically and physiologically significant glucose decreases at the end of the treatment period of 25, 42, 26 and 29% from their Day 0 value, respectively. Untreated animals showed a considerable increase in blood glucose levels during this study. Thus compound **11** exhibited marked antihyperglycemic activity when administered orally for 9 days to the *ob*/*ob* mice and was active in the same range as other compounds of this class. In comparison, oral administration of aqueous solution of compound **4** at a dose of 20 mg/kg for 9 days to *ob*/*ob* mice produced only a 19% decrease in glucose from the Day 0 value (Fig. 2).

In dose range-finding study, *ob*/*ob* diabetic mice were orally administered **11** at three different dose levels (3.1, 6.3, and 12.5 mg/kg body weight) or troglitazone **3** at a dose of 12.5 mg/kg for 19 days. All dose levels of

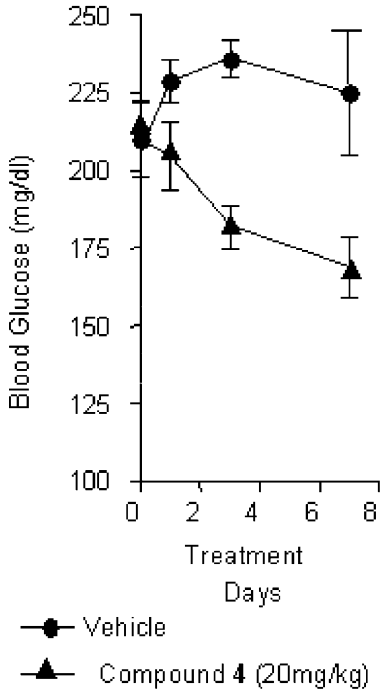


Figure 2. Group mean ± SD blood glucose concentration in *ob*/*ob* mice after administration of **4** or vehicle for 8 days.

Table 2. Percent change in group mean blood glucose compared with control group

Group	Percent decrease time (days)					
	0[a]	3	7	10	15	19
Compound **11** (3.1 mg/kg)	−0.77	16	3	21	25	35
Compound **11** (6.3 mg/kg)	1	37	30	43	40	55
Compound **11** (12.5 mg/kg)	1	49	21	42	40	48
Compound **3** (12.5 mg/kg)	1	44	23	34	30	23

[a]On day 0 all group mean blood glucose were ±1% of vehicle.

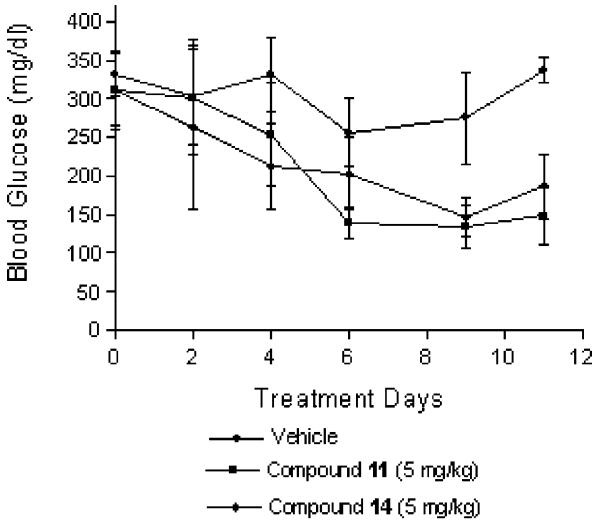


Figure 3. Group mean ± SD blood glucose concentration in *ob*/*ob* mice after administration of **11**, **14** or vehicle for 11 days.

compound **11** were effective in reducing blood glucose at Day 19 by 35, 55, and 47%, from the vehicle group ($p \leq 0.01$, 0.001, 0.001, respectively). A dose–time relationship was evident, and overall blood glucose concentrations were maximally reduced at 6.3 mg/kg. Troglitazone decreased blood glucose by 23–44% over the course of the study. The data is summarized in Table 2. Details of this study and additional biological activities of compound **11** are presented elsewhere.[20]

When compound **11** and its corresponding free acid **14** were orally administered to *ob*/*ob* mice at a dose of 5 mg/kg per day for 11 days, a comparable glucose lowering and normalizing effect was observed for both the groups compared with vehicle-treated controls (Fig. 3). There were no differences in body weights between the two groups over this period. Both the ester **11** and the acid **14** showed similar PPARγ agonist activity and potency (EC_{50} of 0.28 ± 0.14 μM and 0.59 ± 0.17 μM, respectively), and both the compounds showed similar glucose lowering effect. Compound **11** is extensively and rapidly metabolized in mice to compound **14**. In a PK study in male mice dosed orally with compound **11** both **11** and **14** were detected in plasma from 2 min onwards. Terminal half lives of **11** and **14** were 6.5 ± 0.7 and 11.1 ± 4.8 h and plasma $AUC_{0-24\ h}$ of 5.9 ± 1.3 and 85.8 ± 7.5 μg h/mL, respectively. Only 7% of the total mass of compound **11** and **14** was detectable as compound **11** in the blood of male mice.[21] These results indicate rapid conversion of **11** to **14** and support the proposal that **11** is a prodrug of **14** in vivo. The similarity of activity of **11** and **14** as PPARγ agonists and in lowering glucose when administered directly in vitro assays, where there is no conversion of the compounds, together with the metabolism data, suggests that compound **14** is an active metabolite of **11**, which can be therefore be considered an active prodrug. Further, in vitro permeability studies in Caco-2 cells have demonstrated that compound **11** is about 17-fold higher in absorption potential than **14**. Thus compound **11** would be the preferred form for oral administration, compared with **14**, but the net pharmacologic effect would appear to be derived from a combination of both compounds.

Conclusion

We have identified a new series of thiazolidine-1,4-dione substituted α-phenyl cinnamic acids with moderate PPARγ agonist activity showing strong oral glucose lowering effects in animal models of type 2 diabetes. The data suggests that the presence of the cinnamic acid double bond as well as its geometry is very important for PPAR agonism. Thus cinnamic acid based TZDs can provide lead compounds to develop new anti-hyperglycemic agents.

Experimental

Chemistry

General methods. Melting points were measured on a Mel-Temp melting point apparatus and are uncorrected. The ^1H NMR and ^{13}C NMR spectra were recorded on a JEOL Eclipse (400 MHz) or Nicolet NT 36 (360 MHz) spectrometer and are reported as parts per million (ppm) downfield from TMS. The infrared spectra were recorded on a Nicolet Impact 410 FT-IR spectrophotometer. The mass spectra were recorded on a Fison VG Platform II of HP 1100 MDS 1964A mass spectrophotometer. UV spectra were recorded on a Beckman DU650 spectrophotometer. TLC was performed on Merck silica gel F_{254} precoated plates. The silica gel used for column chromatography was 'Baker' silica gel (40 μm) for flash chromatography.

3-(3,5-Dimethoxyphenyl)-2-(4-hydroxyphenyl)-acrylic acid (7). To a mixture of 3,5-dimethoxybenzaldehyde, **5**, (500 g, 3.0 mol) and 4-hydroxyphenylacetic acid, **6**, (457 g, 3.0 mol) was added acetic anhydride (1.0 L, 10.6 mol) and triethylamine (420 mL, 3.0 mol). After stirring at 130–140 °C for 6 h, the mixture was cooled to room temperature. Concentrated HCl (1 L) was added to the reaction mixture slowly over 50 min while keeping the temperature between 20 and 30 °C. The light-yellow precipitate obtained was filtered and washed with water. The solid was dissolved in 3 N NaOH (5 L) and stirred for 1 h and filtered. The filtrate was acidified to pH 1 with concentrated HCl while maintaining a temperature at 25–30 °C. The precipitated product was filtered and washed with water to give crude product that was recrystallized from MeOH–H_2O and dried at 40 °C for

6 h to yield **7** (428 g, 47%): mp 225–227 °C (lit. 226–228 °C);[17] ^{1}H NMR (360 MHz, DMSO-d_6) δ 12.48 (br s, 1H), 9.42 (s, 1H), 7.59 (s, 1H), 6.95 (d, J = 8.0 Hz, 2H), 6.76 (d, J = 8.0 Hz, 2H), 6.35 (t, J = 2.2 Hz, 1H), 6.27 (d, J = 2.2 Hz, 2H), 3.56 (s, 6H); MS (EI) m/z 299 $[M]^-$.

3-(3,5-Dimethoxyphenyl)-2-(4-hydroxyphenyl)-acrylic acid methyl ester (8). Methanol (3.0 L) was added to a thoroughly dried **7** (427.5 g, 1.42 mol) under argon. To this stirred suspension concentrated sulfuric acid (100 mL) was added and heated at reflux for 20 h under nitrogen. Methanol was evaporated under reduced pressure at 30 °C. The residue was taken up in ethyl acetate (3.0 L) and washed with water (2×1.0 L), saturated aqueous $NaHCO_3$ (2×1.0 L), brine (2×1.0 L). The organic layer was dried on anhydrous magnesium sulfate, filtered and the solvent was evaporated. Compound **8** was obtained after drying under high vacuum as white solid, (433.6 g, 97%): mp 106–108 °C; ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.72 (s, 1H), 7.06 (d, J = 7.9 Hz, 2H), 6.77 (d, J = 7.9 Hz, 2H), 6.33 (t, J = 2.2 Hz, 1H), 6.26 (d, J = 2.2 Hz, 2H), 5.74 (s, 1H), 3.81 (s, 3H), 3.60 (s, 6H); MS (EI) m/z 315 $[M]^+$. Anal. ($C_{18}H_{18}O_5$) C, H.

3-(3,5-Dimethoxyphenyl)-2-[4-(4-formylphenoxy)-phenyl]-acrylic acid methyl ester (9). Under argon, compound 8 (433.0 g, 1.37 mol) was dissolved in dry DMF (1.6 L) and sodium hydride (60.4 g, 1.51 mol) was added. To the resulting orange solution, 4-fluorobenzaldehyde (185.0 mL, 1.71 mol) was added and heated at 80 °C for 18 h. The reaction mixture was cooled to room temperature, diluted with ethyl acetate (3.0 L) and extracted with water (3×1.0 L), then brine (1×1.0 L). The organic layer was dried over anhydrous sodium sulfate, filtered and solvent was evaporated. The residue was suspended in methanol (3.0 L), stirred overnight, solid was filtered and dried under vacuum at 40 °C to yield **9** as pale-yellow solid (445 g, 77%): mp 108–110 °C; ^{1}H NMR (360 MHz, $CDCl_3$) δ 9.94 (s, 1H), 7.86 (d, J = 8.6 Hz, 2H), 7.80 (s, 1H), 7.28 (d, J = 8.6 Hz, 2H), 7.11 (overlapped d, J = 9.0 Hz, 2H), 7.08 (overlapped d, J = 9.0 Hz, 2H), 6.36 (t, J = 2.2 Hz, 1H), 6.25 (d, J = 2.2 Hz, 2H), 3.83 (s, 3H), 3.63 (s, 6H). Anal. ($C_{25}H_{22}O_6$) C, H.

3-(3,5-Dimethoxyphenyl)-2-{4-[4-(2,4-dioxothiazolidin-5-ylidenmethyl)-phenoxy]-phenyl}-acrylic acid methyl ester (10). To a stirred suspension of **9** (352 g, 0.82 mol) in anhydrous toluene (2.5 L), 2,4-thiazolidinedione (98.6 g, 0.84 mol), benzoic acid (134 g, 1.10 mol) and piperidine (107.4 g, 1.26 mol) was added sequentially and heated at reflux temperature with continuous removal of water with the help of Dean–Stark apparatus for 5 h. Toluene (1.0 L) was removed from the reaction mixture and cooled overnight at 4°C. The solid that separated was filtered and mother liquor was evaporated to dryness under reduced pressure. The residue obtained was redissolved in a mixture of MeOH–diethyl ether (1 : 1, 3.0 L). On standing overnight at 4 °C, the solution yielded more solids. The solid from both lots were combined and dried overnight in vacuum oven at 40 °C to give **10** as yellow solid (362.5 g, 86%): mp 106–108 °C; ^{1}H NMR (360 MHz, DMSO-d_6) δ 12.53 (br s, 1H), 7.78 (s, 1H), 7.73 (s, 1H), 7.63 (d, J = 9.2 Hz, 2H), 7.25 (d, J = 9.2 Hz, 2H), 7.13 (overlapped d, J = 8.3 Hz, 2H), 7.11 (overlapped d, J = 8.6 Hz, 2H), 6.42 (t, J = 2.2 Hz, 1H), 6.27 (d, J = 2.2 Hz, 2H), 3.73 (s, 3H), 3.59 (s, 6H); MS (EI) m/z 518 $[M]^+$. Anal. ($C_{28}H_{23}NO_7S$) C, H, N.

3-(3,5-Dimethoxyphenyl)-2-{4-[4-(2,4-dioxothiazolidin-5-ylmethyl)phenoxy]-phenyl}-acrylic acid methyl ester (11). To a solution of **10** (599 g, 1.16 mol) in glacial acetic acid (11.5 L), ammonium formate (4.0 kg, 62.9 mol) was added and stirred for 30 min. A slurry of Pd on carbon (10%, dry, 300 g) in glacial acetic acid (500 mL) was added to the flask and heated at 120 °C for 24 h followed by stirring at room temperature for 48 h. The resulting mixture was filtered through a bed of Celite®. The filtrate was poured slowly into vigorously stirred water (12 L) and the solid that separated solid was filtered and dried. The resulting solid was purified by slurring twice in hot methanol and once in hot ethanol to yield **11** as white solid (296 g, 49.2%): mp 126–128 °C; IR (KBr) ν_{max} 3200, 2950, 2850, 1700, 1600, 1500, 1350, 1150, 850 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.01 (br s, 1H), 7.73 (s, 1H), 7.28 (d, J = 8.6 Hz, 2H), 7.19 (d, J = 8.6 Hz, 2H), 7.02 (d, J = 8.6 Hz, 2H), 6.96 (d, J = 8.6 Hz, 2H), 6.40 (t, J = 2.2 Hz, 1H), 6.27 (d, J = 2.2 Hz, 2H), 4.92 (dd, J = 9.2 and 4.4 Hz, 1H), 3.73 (s, 3H), 3.57 (s, 6H), 3.37 (dd, J = 14.8 and 4.3 Hz, 1H), 3.12 (dd, J = 14.8 and 9.4 Hz, 1H); MS (EI) m/z 518 $[M-H]^-$, 265, 249, 113. Anal. ($C_{28}H_{25}NO_7S$) C, H, N.

3-(3,5-Dimethoxyphenyl)-2-{4-[4-(2,4-dioxothiazolidin-5-ylmethyl)-phenoxy]-phenyl}-acrylic acid methyl ester (11). Compound **10** (20 g, 38.6 mmol), ammonium formate (150 g, 2.38 mol), 10% Pt/C (dry, 4 g) and acetic acid (660 mL) were combined into a round-bottom flask equipped with reflux condenser, thermometer and mechanical stirrer. The reactor was evacuated and purged three times with nitrogen then, heated to a steady reflux (ca. 124 °C). Reaction was completed within 15 h and allowed to cool with stirring to ambient room temperature. After cooling, the mixture was filtered though a pad of Celite® (5 g) and the filter pad washed with fresh acetic acid (2×100 mL). The mother liquor and washes were combined and concentrated. The residue was then diluted with dichloromethane (400 mL), and the combined organics were extracted twice with water (400 mL) and 5% bicarbonate (400 mL). The organic portion was then dried and poured through silica gel (30 g) and washed with dichloromethane (2×100 mL). The washes were combined and concentrated. The residue was diluted with ethanol, allowed to cool to 60 °C and seed crystals were added. This slurry was stirred at 50 °C for about 30 min then allowed to cool to ambient room temperature to yield compound **11** (12.85 g, 64%) with an HPLC assay of 98.1%.

3-(3,5-Dimethoxyphenyl)-2[4-(4-hydroxymethylphenoxy)-phenyl]-acrylic acid methyl ester (12). Compound **9** (5.0 g, 11.9 mmol) was suspended in anhydrous ethanol (60 mL) at room temperature and sodium borohy-

dride (0.23 g, 6.1 mmol) was added with efficient stirring. The reaction was followed by TLC and was complete in 1 h, solvent was evaporated and the residue was dissolved in ethyl acetate (100 mL). The organic layer was extracted with water (50 mL), washed with brine (25 mL) brine, dried on anhydrous magnesium sulfate, filtered and solvent was evaporated yield the title compound **12** as white solid (5.1 g, 100%): mp 93–95 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.72 (s, 1H), 7.35 (d, J = 8.8 Hz, 2H), 7.19 (d, J = 8.8 Hz, 2H), 7.02 (d, J = 8.4 Hz, 2H), 7.00 (d, J = 8.4 Hz, 2H), 6.41 (t, J = 2.4 Hz, 1H), 6.29 (d, J = 2.0 Hz, 2H), 5.18 (t, J = 6.4 Hz, 1H), 4.49 (d, J = 4.8 Hz, 2H), 3.73 (s, 3H), 3.57 (s, 6H); MS (EI) m/z 315 $[M]^+$. Anal. ($C_{25}H_{24}O_6$) C, H.

2-[4-(4-Bromomethylphenoxy)-phenyl]- 3-(3,5-dimethoxyphenyl)-acrylic acid methyl ester (13). A solution of PBr_3 (4.8 mL of 1.0 M in CH_2Cl_2) was added dropwise to **12** (5.0 g, 11.9 mmol) dissolved in CH_2Cl_2 (20 mL) at room temperature with good stirring. After 1 h, the solution was extracted with water (2×60 mL) and brine (20 mL). The organic phase was dried over anhydrous magnesium sulfate, filtered through a small bed of silica gel (20 g) and solvent was evaporated. The resulting tacky syrup was dried under high vacuum for 48 h at room temperature to yield compound **13** (5.7 g, 99.0%): mp 79–81 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.73 (s, 1H), 7.49 (d, J = 8.4 Hz, 2H), 7.22 (d, J = 8.4 Hz, 2H), 7.07 (d, J = 8.4 Hz, 2H), 7.00 (d, J = 8.4 Hz, 2H), 6.42 (t, J = 2.4 Hz, 1H), 6.28 (d, J = 2.0 Hz, 2H), 4.73 (d, J = 4.8 Hz, 2H), 3.68 (s, 3H), 3.58 (s, 6H). Anal. ($C_{25}H_{23}BrO_5$) C: calcd 61.12; found 62.26.

3-(3,5-Dimethoxyphenyl)-2-{4-[4-(2,4-dioxothiazolidin-5-ylmethyl)phenoxy] phenyl}-acrylic acid methyl ester (11). 2,4-Thiazolidinedione (2.83 g, 24.2 mmol) was dissolved in dry THF (170 mL) and cooled to 0 °C under argon. Butyllithium (1.6 M in hexanes, 30 mL, 48.0 mmol) was added dropwise and stirring was continued for 0.5 h at 0 °C. Under argon, a solution of **13** (5.7 g, 11.8 mmol) in dry THF (30 mL) was added rapidly via syringe to the reaction mixture with rapid stirring. The temperature was maintained at 0 °C for 45 min before quenching with aqueous HCl (5%, 40 mL). Additional H_2O (40 mL) was added and the mixture was extracted with ethyl acetate (3×30 mL). The organic layers were combined, washed with brine, dried over anhydrous magnesium sulfate, filtered and the solvent was evaporated. Flash chromatography over silica gel using hexanes-ethyl acetate (3:2) as eluting solvent yielded the title compound, **11**, (0.93 g, 15%). Melting point and ^{1}H NMR of compound **11** made by this method was identical with **11** produced from **10**.

3-(3,5-Dimethoxyphenyl)-2-{4-[4-(2,4-dioxothiazolidin-5-ylmethyl)-phenoxy]-phenyl acrylic acid (14). To a stirred, cooled below 10 °C, suspension of **11** (10 g, 19.27 mmol) in methanol (50 mL), aqueous sodium hydroxide (2 N, 33.7 mL, 67.4 mmol) was added and stirring was continued for 15 h at room temperature. The resulting pale-yellow solution was cooled to 10 °C and acidified with aqueous HCl (5%, 115 mL). The solid that separated was filtered and washed with water (3×30 mL), and recrystallized from ethanol to give **14** as white solid (7.14 g, 73%): mp 138–140 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.69 (s, 1H), 7.28 (d, J = 8.8 Hz, 2H), 7.19 (d, J = 8.8 Hz, 2H), 7.02 (d, J = 8.8 Hz, 2H), 6.97 (d, J = 8.8 Hz, 2H), 6.41 (t, J = 2.4 Hz, 1H), 6.28 (d, J = 2.4 Hz, 2H), 4.92 (dd, J = 9.2 and 4.4 Hz, 1H), 3.58 (s, 6H), 3.38 (dd, J = 14.0 and 4.0 Hz, 1H), 3.13 (dd, J = 14.4 and 9.2 Hz, 1H); MS (EI) m/z 506 $[M]^+$. Anal. ($C_{27}H_{23}NO_7S$) C, H.

3-(3,5-Dimethoxyphenyl)-2-(4-hydroxyphenyl)-propionic acid methyl ester (15). To a suspension of **8** (6.28 g, 20.0 mmol) in ethanol (200 mL), palladium on carbon (10%, wet, 0.63 g) was added and the mixture was stirred under H_2 at atmospheric pressure at room temperature for 18 h. The catalyst was filtered through a bed of Celite® and solvent was evaporated under reduced pressure to yield **15** as white solid (6.32 g, 100%): mp 63–65 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.15 (d, J = 8.7 Hz, 2H), 6.74 (d, J = 8.7 Hz, 2H), 6.29 (t, J = 2.4 Hz, 1H), 6.25 (d, J = 2.4 Hz, 2H), 3.78 (t, J = 8.7 Hz, 1H), 3.72 (s, 6H), 3.62 (s, 3H), 3.31 (dd, J = 13.5 and 8.4 Hz, 1H), 2.93 (dd, J = 13.5 and 6.9 Hz, 1H). MS (EI) m/z 317 $[M]^+$. Anal. ($C_{18}H_{20}O_5$) C, H.

3 - (3,5 - Dimethoxyphenyl) - 2 - [4 - (4 - formylphenoxy) - phenyl]-propionic acid methyl ester (16). To a suspension of sodium hydride (60% in oil, 0.25 g, 6.3 mmol) in DMF (2 mL) under argon, **15** (2.0 g, 6.3 mmol) in dry DMF (3 mL) was added. To the resulting yellow solution, 4-fluorobenzaldehyde (0.68 mL, 6.3 mmol) was added and heated at 80 °C for 18 h. The reaction mixture was cooled to room temperature and water (20 mL) was added and extracted with ethyl acetate (3×50 mL). The organic layer was dried over anhydrous sodium sulfate, filtered and the solvent was evaporated. The ethyl acetate solution of crude product was filtered through a small bed of silica gel to yield **16** (1.83 g, 69%) as oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.91 (s, 1H), 7.84 (d, J = 8.7 Hz, 2H), 7.33 (d, J = 8.7 Hz, 2H), 7.04 (d, J = 5.4 Hz, 2H), 7.01 (d, J = 5.4 Hz, 2H), 6.30 (t, J = 2.1 Hz, 1H), 6.25 (d, J = 2.1 Hz, 2H), 3.86 (t, J = 7.8 Hz, 1 Hz), 3.76 (s, 6H), 3.66 (s, 3H), 3.36 (dd, J = 12.6 and 8.1 Hz, 1H), 2.97 (dd, J = 13.5 and 7.5 Hz, 1H). MS (EI) m/z 421 $[M]^+$.

3-(3,5-Dimethoxyphenyl)-2-{4-[4-(2,4-dioxothiazolidin-5-ylidenmethyl)-phenoxy]-phenyl}-propionic acid methyl ester (17). To a stirred suspension of **16** (1.81 g, 4.3 mmol) in anhydrous toluene (25 mL), 2,4-thiazolidinedione (0.56 g, 4.74 mmol), benzoic acid (0.68 g, 5.60 mmol) and piperidine (0.60 mL, 6.03 mmol) were added sequentially and heated at reflux temperature with continuous removal of water (Dean–Stark apparatus) for 2 h. The solvent was evaporated to dryness under reduced pressure the residue was purified by silica gel chromatography eluting with hexane–ethyl acetate (1:1) to yield **17** (1.82 g, 81%): mp 104–106 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.53 (br s, 1H), 7.76 (s, 1H), 7.60 (d, J = 8.7 Hz, 2H), 7.35 (d, J = 8.7 Hz, 2H), 7.07 (d, J = 4.8 Hz, 2H), 7.03 (d, J = 4.8 Hz, 2H), 6.33–6.28 (m, 3H), 4.01 (t, J = 7.5 Hz, 1 Hz), 3.66 (s, 6H), 3.56 (s, 3H), 3.22 (dd, J = 13.8 and 8.4 Hz, 1H), 2.90 (dd, J = 13.5 and 7.2

Hz, 1H). MS (EI) *m/z* 520 $[M]^+$. Anal. ($C_{28}H_{25}NO_7S$) C: calcd 64.73; found 65.89.

3-(3,5-Dimethoxyphenyl)-2-{4-[4-(2,4-dioxothiazolidin-5-ylmethyl)-phenoxy]-phenyl}-propionic acid methyl ester (18). Compound **17** (1.6 g, 3.08 mmol) was dissolved in dioxane (45 mL) and transferred in a hydrogenation bottle and Pd on carbon (10%, 1.0 g) was added. Hydrogenation was done at 65 psi for 34 h. Following this period, additional Pd on carbon (10%, 0.6 g) was added and hydrogenation was allowed to continue for another 18 h. Catalyst was filtered through a bed of Celite® and solvent was evaporated. The residue was purified by column chromatography on reverse phase silica gel (C-18) using acetonitrile–water (1:1) mixture to elute **18** as white solid (0.60 g, 38%): mp 125–128 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.04 (br s, 1H), 7.31 (d, J = 8.4 Hz, 2H), 7.25 (d, J = 8.4 Hz, 2H), 6.92 (d, J = 8.6 Hz, 4H), 6.30 (d, J = 2.0 Hz, 2H), 6.29 (t, J = 2.0 Hz, 1H), 4.90 (dd, J = 9.2 and 4.4 Hz, 1H), 3.98 (t, J = 8.0 Hz, 1H), 3.67 (s, 6H), 3.56 (s, 3H), 3.37 (dd, J = 13.6 and 4.0 Hz, 1H), 3.21 (dd, J = 14.0 and 8.8 Hz, 1H); 3.11 (dd, J = 14.0 and 9.2 Hz, 1H), 2.90 (dd, J = 13.6 and 7.6 Hz, 1H). MS (EI) *m/z* 522 $[M]^+$. Anal. ($C_{28}H_{27}NO_7S$) C, H, N.

***Z*-3-(3,5-Dimethoxyphenyl)-2-(4-hydroxyphenyl)-acrylic acid (19).** *E*-3-(3,5-Dimethoxyphenyl)-2-(4-hydroxyphenyl)-acrylic acid, **7** (10.0 g, 0.33 mmol) was dissolved in a mixture of acetic anhydride (40 mL, 0.42 mol) and triethylamine (40 mL, 0.29 mol) and heated at 125 °C for 24 h.[19] The mixture was cooled to room temperature and ethyl acetate (150 mL) was added, the mixture was cooled to 5 °C, acidified with concentrated HCl (30 mL) and stirred for 90 min. The organic layer was separated and the aqueous layer was extracted with ethyl acetate (3×100 mL). The combined organic layers were washed with water (2×50 mL) and extracted with aqueous NaOH (5M, 3×70 mL). The aqueous alkaline layer was acidified with glacial acetic acid (65 mL) to pH 5.2 and stirred at 0 °C for 30 min. The solid that separated was filtered and mother liquor was acidified with concentrated HCl (90 mL) and stirred at 5°C for 1 h. The solid that separated was filtered, washed with cold water (2 °50 mL) and dried at 45 °C for 6 h to yield, **19**, (1.3 g, 13%): mp 135–137 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.28 (br, 1H), 9.70 (br, 1H), 7.32 (d, J = 10.4 Hz, 2H), 6.81(s, 1H, overlapped), 6.79 (d, J = 9.7 Hz, 2H), 6.67 (d, J = 2.5 Hz, 2H), 6.64 (t, J = 2.5 Hz, 1H), 3.73 (s, 6H). MS (EI) *m/z* 299 $[M]^-$.

***Z*-3-(3,5-Dimethoxyphenyl)-2-(4-hydroxyphenyl)-acrylic acid methyl ester (20).** Concentrated sulfuric acid (10 drops) was added to a stirred methanol suspension of thoroughly dried **19** (0.60 g, 2.0 mmol) under argon and heated at reflux for 18 h. Methanol was evaporated under reduced pressure, the residue was taken up in ethyl acetate (20 mL) and washed with water (20 mL), saturated aq $NaHCO_3$ (10 mL) and brine (10 mL). The organic layer was dried on anhydrous magnesium sulfate, filtered and the solvent was evaporated. The crude product obtained was purified by chromatography over silica gel and eluted with hexane–ethyl acetate (7:3) to yield **20** as white solid (0.24 g, 35%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 (d, J = 8.4 Hz, 2H), 6.82 (s, 1H), 6.76 (d, J = 8.4 Hz, 2H), 6.45 (d, J = 2.0 Hz, 2H), 6.34 (t, J = 2.0 Hz, 1H), 4.97 (s, 1H), 3.73 (s, 3H), 3.72 (s, 6H).

***Z*-3-(3,5-Dimethoxyphenyl)-2-[4-(4-formylphenoxy)-phenyl]-acrylic acid methyl ester, (21).** Under argon, compound **20** (0.60 g, 1.9 mmol) was dissolved in dry DMF (4 mL) and sodium hydride (60% in oil, 0.09 g, 2.28 mmol) was added. To the resulting orange solution, 4-fluorobenzaldehyde (0.25 mL, 2.28 mmol) was added and heated at 80 °C for 18 h. The reaction mixture was cooled to room temperature and water (10 mL) was added. The mixture was extracted with ethyl acetate (3×20 mL) and the crude product obtained after evaporation was purified by chromatography over silica gel and elution with a mixture of hexane–ethyl acetate (4:1) to yield **21** as white solid (0.59 g, 74%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.93 (s, 1H), 7.86 (d, J = 8.8 Hz, 2H), 7.49 (d, J = 8.8 Hz, 2H), 7.10 (overlapped d, J = 8.8 Hz, 2H), 7.08 (overlapped d, J = 8.8 Hz, 2H), 6.96 (s, 1H), 6.53 (dd, J = 2.8 Hz, 2H), 6.43 (t, J = 2.0 Hz, 1H), 3.80 (s, 3H), 3.79 (s, 6H).

***Z*-3-(3,5-Dimethoxyphenyl)-2-{4-[4-(2,4-dioxothiazolidin-5-ylidenmethyl)-phenoxy]-phenyl}-acrylic acid methyl ester (22).** To a stirred suspension of **21** (0.53 g, 1.3 mmol) in anhydrous toluene (10 mL), 2,4-thiazolidinedione (0.15 g, 1.30 mmol), benzoic acid (0.21 g, 1.69 mmol) and piperidine (0.19 g, 1.26 mol) were added sequentially and the mixture was heated at reflux temperature with continuous removal of water (Dean–Stark apparatus) for 5 h. Toluene was evaporated and the residue was chromatographed over silica gel eluting with hexane–ethyl acetate (1:1) to yield a mixture of **22** and **10** (0.60 g, 91%) in a ratio of 7:1 on the basis of proton NMR analysis. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.53 (br s, 1H), 7.79 (s, 1H), 7.65 (d, J = 8.8 Hz, 2H), 7.54 (d, J = 8.8 Hz, 2H), 7.18 (overlapped d, J = 8.8 Hz, 2H), 7.16 (overlapped d, J = 8.8 Hz, 2H), 7.17 (overlapped s, 1H), 6.57 (d, J = 2.0 Hz, 2H), 6.50 (t, J = 2.0 Hz, 1H), 3.79 (s, 3H), 3.75 (s, 6H).

***Z*-3-(3,5-Dimethoxyphenyl)-2-{4-[4-(2,4-dioxothiazolidin-5-ylmethyl)-phenoxy]-phenyl}-acrylic acid methyl ester (23).** To a solution of **22** (0.60 g, 1.6 mmol) in acetic acid (15 mL) was added Pd on carbon (10%, 300 mg) and ammonium formate (4.3 g, 55.8 mmol) and the mixture was heated at 120 °C for 20 h. Catalyst was filtered through a bed of Celite® and acetic acid was evaporated under reduced pressure. Water (50 mL) was added to the residue and solid separated was filtered. Pure *Z*-isomer **23** was isolated by preparative HPLC using Intersil ODS-3 preparative column (250×4.6 mm, 5 μm) running at a rate of 15 mL per min using methanol/acetonitrile/water (3:3:2) containing formic acid (0.05%), purity 99.0%, (0.09 g, 15.0%): mp 65–66 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.05 (br s, 1H), 7.48 (d, J = 9.2 Hz, 2H), 7.29 (d, J = 8.4 Hz, 2H), 7.13 (s, 1H), 7.03 (overlapped d, J = 8.8 Hz, 2H), 7.01 (overlapped d, J = 8.4 Hz, 2H), 6.56 (d, J = 2.0 Hz, 2H), 6.49 (t, J = 2.0 Hz, 1H), 4.90 (dd, J = 9.2 and 4.4 Hz, 1H),

3.77 (s, 3H), 3.75 (s, 6H), 3.38 (dd, $J=14.8$ and 4.8 Hz, 1H), 3.13 (dd, $J=14.4$ and 9.2 Hz, 1H). MS (EI) m/z 518 $[M-H]^-$.

Pioglitazone **1**, rosiglitazone, **2**, and troglitazone, **3**, used were isolated by purification of commercially available Actose®, Avandia® and Rezulin® respectively. Purity and identity of all the three compounds were checked by HPLC and ^1H NMR.

Biology

Transfection and luciferase activity assay. Human PPARγ2 expression vector was constructed by inserting PPARγ2 encoding region into pcDNA3.1+ vector (Invitrogen, Carlsbad, CA, USA). Luciferase reporter vector was constructed by ligating PPRE response element upstream of the Firefly luciferase coding region. Control vector pRL-SV40 expressing Renilla luciferase was purchased from Promega (Madison, WI, USA).

About 2.7×10^4 HEK293 cells (ATCC, Manassas, VA, USA) were plated into a 35 mm culture well and maintained in Eagle's Minimal Essential Medium (EMEM, ATCC, Manassas, VA, USA) supplemented with 10% heat inactivated horse serum (ATCC, Manassas, VA, USA) for 24 h. Expression, reporter and control vectors (2.5 ng control and 100 ng others per culture well) were transfected by Lipofectamin Plus™ Reagent (Invitrogen, Carlsbad, CA, USA). Transfection reagent and DNA were prepared according to manufacture's recommendations and incubated with cells for 3 h followed by addition of an equal volume of EMEM supplemented with 20% horse serum. Twenty-four h after transfection, cells were treated with vehicle or compounds at indicated final concentration for 24 h. Final concentration of DMSO was 0.01% in medium. Vehicle and compound treatment were all conducted in triplicate. Each culture well was then assayed for a response characterized by increased Firefly luciferase activity normalized with Renilla luciferase activity.

Assays for Firefly luciferase activity and Renilla luciferase activity followed the standard protocol of Dual-luciferase Reporter® Assay System (Promega, Madison, WI, USA). Briefly, 400 μL Passive Lysis Buffer was added into each culture well and all wells were placed on a shaker for 15 min. Cell lysate (5 μL) of each well was added to a reaction tube. Luciferase reagent II and Stop & Glo® were injected into the reaction tube sequentially by Sirius Luminometer (Berthold Detection Systems, Pforzheim, Germany). Final reporter activity was calculated as the ratio of Firefly luciferase activity over Renilla luciferase activity.

Caco-2 permeability. Caco-2 permeability studies were done by Absorptions System, Exton following standard protocol and analyzing samples by mass spectrometer with electrospray interface and single ion monitoring mode.

In vivo studies. All procedures performed were in compliance with the Animal Welfare Act and US Department of Agriculture regulations and were approved by the Calyx Therapeutics Institutional Animal Care and Use Committee. Animals were housed at 22 °C and 50% relative humidity, with a 12-h light and dark cycle, and received a regular rodent diet (Harlan Teklad, Madison, WI, USA) ad libitum with free access to water. Male C57BL/6J-*ob*/*ob* mice were obtained from Jackson Laboratories (Bar Harbor, ME, USA) when their age was 5 weeks. Groups of 5–10 7–8-week-old animals were orally dosed with suspensions of test compounds, or vehicle, 0.5% carboxymethyl cellulose (CMC, Sigma, St. Louis, MO, USA) in water or phosphate-buffered saline (PBS), in some cases containing PEG-400 (10%), once daily by gavage. Body weights were recorded periodically, and animals were observed daily. Blood glucose measurements were made with One Touch Glucose Meter (Life Scan, Inc., Milpitas, CA, USA) prior to administering the next dose or 24 h after the last dose and in the fed state.

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