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Synthesis and SAR of thiazolidinedione derivatives as 15-PGDH inhibitors

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ABSTRACT

Prostaglandins have a short life in vivo because they are metabolized rapidly by oxidation to 15-keto-prostaglandins catalyzed by a cytosolic enzyme known as NAD$^+$-dependent 15-hydroxyprostaglandin dehydrogenase (15-PGDH). Previously, **CT-8**, a thiazolidinedione analogue, was found to be a potent inhibitor of 15-PGDH. Structure–activity analysis indicated that the N-methylation of thiazolidine-2,4-dione, **CT-8**, abolished the inhibitory activity, whereas the introduction of an ethyl hydroxyl group at amine in **CT-8** still had a good inhibitory effect. Based on the structures of the thiazolidinediones analogues and inhibitory activity, a range of benzylidene thiazolidinedione derivatives were synthesized with different substituents on the phenyl ring and their inhibitory activity was evaluated. Replacement of the cyclohexylethyl group of **CT-8** with the hetero five-member ring increased the inhibitory potency. However, replacement of the cyclohexylethyl group with a hetero six-member ring decreased the inhibitory potency significantly. It was found that compound **2** (5-(4-(2-(thiophen-2-yl)ethoxy)benzylidene)thiazolidine-2,4-dione) was the most potent inhibitor that was effective in the nanomolar range.

1. Introduction

Prostaglandins are derived from arachidonic acid through the cyclooxygenase (COX) pathway. Two COX isoforms have been recognized. COX-1 is expressed constitutively in various tissues, including the stomach, whereas COX-2 is induced by cytokines, growth factors, tumor promoters and other agents. Prostaglandins have a short life time in vivo because they are metabolized rapidly by oxidation to 15-ketoprostaglandins catalyzed by a cytosolic enzyme named NAD$^+$-dependent 15-hydroxyprostaglandin dehydrogenase (15-PGDH).[1] This enzyme is present ubiquitously in mammalian tissues and is responsible for the biological inactivation of prostaglandins because 15-ketoprostaglandins possess significantly lower biological activities.[2] Two different types of 15-PGDH have been recognized. Type I is NAD$^+$ specific, while Type II is NADP$^+$ preferred. Type I is more prostaglandin specific and exhibits a low K_m for prostaglandins, whereas Type II has a much broader substrate specificity and shows a high K_m for prostaglandins.[3] Indeed, Type II was later found to be identical to carbonyl reductase.[4] Therefore, Type I is considered to be the key enzyme responsible for the biological inactivation of prostaglandins. Studies on the prostaglandin catabolism have focused on the Type I enzyme (hereafter referred to as 15-PGDH).

Prostaglandins have been implicated in a wide variety of physiological and pathological processes. Prostaglandin E_2 (PGE_2) is a major mediator of inflammation and a key player in the control of various physiological functions. Recently, clinical studies demonstrated that PGE_2 causes the growth of body hair and eyelashes in humans and animals.[5] In humans, trials carried out on the scalp have shown that PGE_2 could increase the hair density.[6] Furthermore, 15-PGDH is also expressed in hair melanocytes. Inhibition of this enzyme as a target to reduce hair loss has been reported.[7] PGE_2 has also been identified as an important mediator of gastric ulcer healing,[8–16] bone formation,[17–21] and dermal wound healing.[22–27] Therefore, inhibitors of 15-PGDH will be valuable for the therapeutic management of such disorders.

Previously, it was reported that ciglitazone, an antidiabetic thiazolidinedione, is a potent antagonist of the 15-PGDH enzymatic activity with an IC_{50} of 2.7 μM.[28] In addition, the inhibitory potency of ciglitazone was higher than those of other thiazolidinediones, such as rosiglitazone (10 times) and troglitazone (127 times) (Fig. 1). Structure–activity analysis of thiazolidinediones also indicated that the nature of the moiety linking to benzylidenethiazolidine-2,4-dione through an ether linkage plays an important role in its inhibitory potency.[29] Based on the structures of the thiazolidinediones analogues and inhibitory activity, various benzylidene thiazolidinedione derivatives with different substituents on the

Abbreviations: 15-PGDH, NAD$^+$-dependent 15-hydroxyprostaglandin dehydrogenase; DTT, dithiothreitol; SDS, sodium dodecylsulfate; EDTA, ethylenediamine-*N,N,N′,N′*-tetraacetic acid; GST, glutathione *S*-transferase; THF, tetrahydrofuran.

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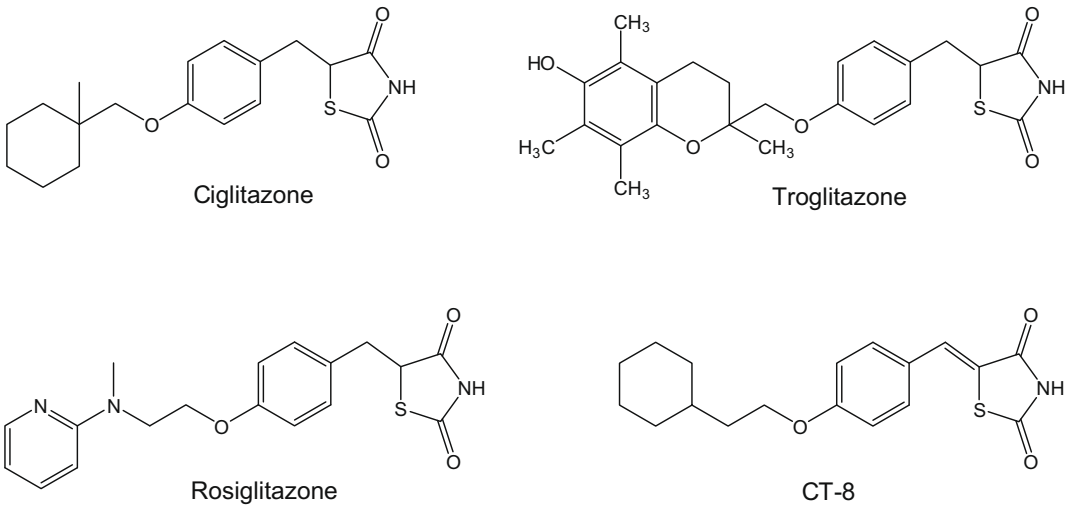


Figure 1. Structures of ciglitazone, rosiglitazone, troglitazone, and **CT-8**.

phenyl ring were synthesized using a series of reactions and tested for the 15-PGDH inhibitory activity.

2. Results and discussion

PGE_2 is a major inflammatory product derived from arachidonic acid through the cyclooxygenase pathway, which is involved in pain and inflammatory responses and is a key player in controlling various physiological functions. Many studies have reported that PGE_2 is an important mediator of dermal wound healing with specific effects on fibroblast behavior. Of particular interest is the work by Kolodsick et al.,[30] who reported that PGE_2 can inhibit the differentiation of fibroblasts into myofibroblasts via the EP2 receptor pathway through the up-regulation of cAMP.

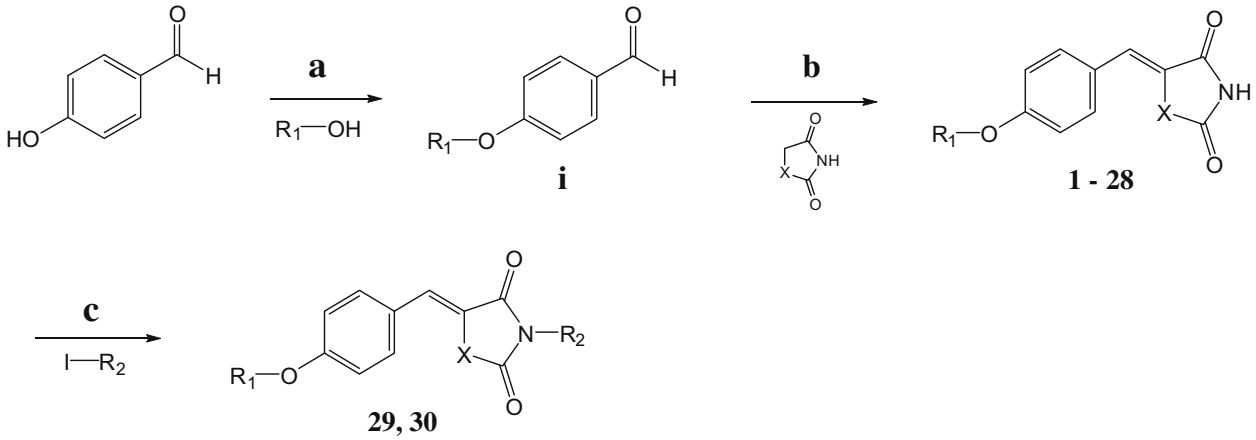
PGE_2 has been also identified as an important mediator of gastric ulcer healing. Hatazawa et al.[31] reported that endogenous PGE_2 plays a role in the healing of NSAID-induced intestinal ulcers through the EP4 receptors. They also reported that the healing–promoting action of PGE_2 is associated with an increase in angiogenesis by upregulating VEGF expression in the fibroblasts of the gastric ulcer bed or margin by activating the EP4 receptors.[8] Numerous in vivo studies have also identified PGE_2 as a potent anabolic agent that stimulates both modeling (i.e., formation drift on quiescent surface) and remodeling-dependent (i.e., positive basic multicellular unit bone balance) bone gain when delivered intermittently by daily subcutaneous injections.[32–40] Prostaglandins, including PGE_1, PGE_2, and $PGF_{2\alpha}$, have been demonstrated to stimulate both bone resorption and bone formation but tend to favor bone formation, thereby increasing bone mass and bone strength.[20,21] Endogenous PGE_2 increases locally after fracture and the inhibition of PGE_2 production impairs bone healing.[41,42] In contrast, the local administration of PGE_2 stimulates bone formation and callus development in animal models.[22]

15-PGDH catalyzes the NAD^+ dependent oxidation of the 15(*S*)-hydroxyl group of prostaglandins and is considered to be a key enzyme in the biological inactivation of prostaglandins. Therefore, inhibitors of 15-PGDH will be valuable for the therapeutic management of diseases requiring elevated PGE_2 levels.

Scheme 1 summarizes the general synthetic routes of thiazolidine-2,4-dione derivatives. *p*-Hydroxybenzaldehyde, as a starting material, was reacted with various substituents to afford the substituted benzaldehyde intermediate in good yield. The intermediate obtained was then used for a coupling reaction with thiazolidine-2,4-dione to afford the appropriate thiazolidine-2,4-dione derivatives. All the synthesized compounds were assayed in vitro against 15-PGDH. Table 1 lists their inhibitory activities (IC_{50} values).

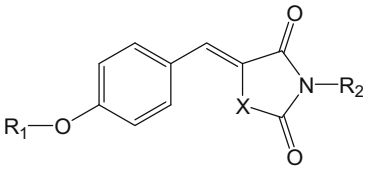
Previously, it was reported that ciglitazone and its derivatives are potent inhibitors of 15-PGDH. This indicates that the benzylidene thiazolidine-2,4-dione analog showed significantly higher inhibitory potency than the benzyl thiazolidine-2,4-dione analog. It was also interesting to discover that the amine group of thiazolidine-2,4-dione plays an important role in the inhibitory potency. This was further confirmed by the synthesis of another N-methylated derivative (compound **29**), in which protection of the amine group in the molecule by methylation rendered the compound totally inactive. In order to demonstrate the role of the amine group in the molecule, an ethyl hydroxyl group was introduced instead of hydrogen and the inhibitory potency of the compounds was compared. Interestingly, the introduction of an ethyl hydroxyl group at the amine group in **CT-8** still produced a good inhibitory effect. This suggests that the hydrogen bond donating groups of thiazolidine-2,4-dione are essential to orient the molecule more favorably toward the binding site in the enzyme. Further structure–activity analysis indicated that the replacement of S in thiazolidine-2,4-dione with NH or CH_2 decreased the inhibitory potency of **CT-8** significantly, as shown in Table 1. Replacement of the cyclohexylethyl group with the hetero five-member ring (compounds **2** and **4**) increased the inhibitory potency. However, replacement of the cyclohexylethyl group with the hetero six-member ring decreased the inhibitory potency significantly, as indicated for compounds **7**, **9**, **11**, and **16**. The most potent inhibitor of this series of compounds was compound **2** (5-(4-(2-(thiophen-2-yl)ethoxy)benzylidene)thiazolidine-2,4-dione) with an IC_{50} of 0.031 μM. Table 1 summarizes the inhibitory potency of all these compounds.

Many studies have reported that the local administration of PGE_2 accelerates the healing of gastric ulcers and wounds, and increases bone formation and callus development in animal models. However, local administration of PGE_2 is an unacceptable therapeutic option for human diseases due to the limited knowledge of the potential changes caused by it on the tissue and cellular level as well as the biological instability of PGE_2. Therefore, inhibitors of 15-PGDH will be valuable for the therapeutic management of diseases requiring elevated prostaglandin levels.



Scheme 1. Reagents and conditions: (a) PPh_3, DEAD, THF, 25 °C, 18 h (88%); (b) piperidine, AcOH, reflux, 12 h (90%); (c) NaH, THF, 25 °C, 3 h (85%).

Table 1
Inhibitory potency of the various synthetic thiazolidine-2,4-diones



Compound	R_1	R_2	X	IC_{50} (μM)
CT-8	Cyclohexylethyl	H	S	0.051
1	2-Thiomorpholine-1,1-dioxideethyl	H	S	0.274
2	2-(Thiophen-2-yl)ethyl	H	S	0.031
3	2-Morpholinoethyl	H	S	0.713
4	2-(Thiophen-3-yl)ethyl	H	S	0.060
5	2-Isopropoxyethyl	H	S	1.248
6	2-(Pyridin-2-yl)ethyl	H	S	0.660
7	2-(Cyclohexylamino)ethyl	H	S	3.719
8	2-(Tetrahydro-2*H*-pyran-2-yl)ethyl	H	S	0.750
9	2-(Piperidin-1-yl)ethyl	H	S	1.442
10	2-(4-Methylthiazol-5-yl)ethyl	H	S	0.636
11	3-Thiomorpholine-1,1-dioxidepropyl	H	S	1.960
12	Thiophen-2-ylmethyl	H	S	0.287
13	Thiophen-3-ylmethyl	H	S	0.429
14	2-Cyclopentylethyl	H	S	0.116
15	Furan-2-ylmethyl	H	S	0.893
16	Pyridin-2-ylmethyl	H	S	2.585
17	4-Methoxybenzyl	H	S	0.529
18	4-Methylbenzyl	H	S	0.232
19	Benzo[*d*][1,3]dioxol-5-ylmethyl	H	S	0.252
20	Cyclopentylmethyl	H	S	0.045
21	4-(Chloromethyl)benzyl	H	S	0.124
22	(4-Methylcyclohexyl)methyl	H	S	0.052
23	2-(Cyclohexyloxy)ethyl	H	S	0.219
24	(2,3-Dihydrobenzo[*b*][1,4]dioxin-2-yl)methyl	H	S	0.172
25	(4-Methyloxycarbonylcyclohexyl)methyl	H	S	0.620
26	Biphenyl-4-ylmethyl	H	S	0.814
27	Cyclohexylethyl	H	NH	0.865
28	Cyclohexylethyl	H	CH_2	3.789
29	Cyclohexylethyl	CH_3	S	ND
30	Cyclohexylethyl	CH_2CH_2OH	S	0.526

The enzyme was assayed fluorometrically as described in Section 3. The IC_{50} value was determined using NAD^+ (250 μM) as coenzyme and PGE_2 (21 μM) as substrate. 15-PGDH was expressed as a GST fusion enzyme using pGEX-2T vector as described in Section 3. ND: No detectable activity.

3. Experimental procedures

3.1. Materials

PGE_2, NAD^+, NADH, Glutathione–Sepharose 4B, dithiothreitol (DTT), sodium dodecylsulfate (SDS), EDTA, and reduced glutathione were obtained from Sigma. The GST gene fusion pGEX-2T expression vector was purchased from Pharmacia Corp. The cDNA of human 15-PGDH was cloned from a human placenta cDNA library, as described previously.[43] All chemical reagents were commercially available. The UV spectra were obtained using a UV–vis spectrophotometer (SHIMADZU). The TLC plates were prepared

using Kieselgel 60 PF254. Column chromatography was performed using silica gel (230–400 mesh, Whatman Inc.). The NMR spectra were recorded on a JEOL JNM-LA 300 spectrometer (JOEL, Tokyo, Japan). The chemical shifts are reported in parts per million (δ) and the signals are quoted as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet). Various thiazolidinediones were synthesized using published procedures.[44]

3.2. Expression and purification of 15-PGDH

The sequence of 15-PGDH cDNA plasmid containing BamHI and EcoRI sites of the pGEX-2T expression vector was used to transform *Escherichia coli* BL-21 LysS. The cells were grown in 500 mL LB medium containing 50 μg/mL ampicillin at 37 °C and 220 rpm until the OD_{600} reached 0.6. Isopropyl β-D-thiogalactoside (1 mM) was added and the cells were allowed to grow for 12 h at 25 °C. The cells were then harvested by centrifugation at 4000*g* for 30 min at 4 °C. The cell pellets was resuspended in 20 mL of a cold cell lysis buffer [1× PBS buffer (pH 7.4) containing 1 mM EDTA and 0.1 mM DTT] and sonicated (14 × 10 s at 4 °C). The disrupted cells were centrifuged at 4000*g* for 20 min at 4 °C. The supernatant was applied slowly to a Glutathione–Sepharose 4B column (approximately 3 mL), which was equilibrated at 4 °C with a lysis buffer [1× PBS buffer (pH 7.4) containing 1 mM EDTA and 0.1 mM DTT]. The lysis buffer was washed until the OD_{280} reached <0.005. The 15-PGDH was eluted from the Glutathione–Sepharose 4B column by incubation at room temperature for 5 min with the elution buffer [50 mM Tris–HCl (pH 8.0) containing 10 mM reduced glutathione, 1 mM EDTA and 0.1 mM DTT]. The concentration of the purified enzyme was determined and the purity of the 15-PGDH was assessed by SDS–PAGE.

3.3. 15-PGDH assay

Assays for the activity of the 15-PGDH inhibitors were performed using a fluorescence spectrophotometer by measuring the formation of NADH at 468 nm following excitation at 340 nm. 50 mM Tris–HCl (pH 7.5), 0.1 mM DTT, 0.25 mM NAD^+, 10 μg of the purified enzyme, 21 μM PGE_2, and various concentrations of inhibitors (total 2 mL) were added to the cell. Each concentration was assayed in triplicate. The absorbance of the reaction mixture was read at 340 nm and the activity of the 15-PGDH inhibitors was determined from a standard curve prepared from the absorbance of various concentrations of NADH at 340 nm.

3.4. General procedure for the synthesis of compounds 1–28

Diethyl azodicarboxylate (40% in toluene, 2.7 g, 6.2 mmol) was added slowly to a stirring solution of 4-(2-hydroxyethyl)thiomorpholine 1,1-dioxide (1 g, 5.6 mmol), *p*-hydroxybenzaldehyde (684 mg, 5.6 mmol) and triphenylphosphine (1.62 g, 6.2 mmol) in THF (20 mL) for 10 min. at 0 °C. The mixture was then stirred at room temperature until the starting materials (TLC analysis) began to disappear. The resulting solution was concentrated under reduced pressure and purified by column chromatography over silica gel (elution with hexane/ethyl acetate, 2:1) to afford 1.37 g of the intermediate, 4-(2-thiomorpholine 1,1-dioxideethoxy)benzaldehyde (87%), as a yellow oil. A mixture of 4-(2-thiomorpholine 1,1-dioxideethoxy)benzaldehyde (1 g, 3.5 mmol), 2,4-thiazolidinedione (410 mg, 3.5 mmol), piperidine (0.17 mL, 1.75 mmol) and acetic acid (0.10 mL, 1.75 mmol) in toluene (20 mL) was then placed into a round bottom flask fitted with a Dean–Stark water trap and stirred overnight under reflux. After cooling to room temperature, the precipitate was washed with hexane and dried to afford compound **1**.

3.4.1. 5-[4-(2-Thiomorpholine-1,1-dioxideethoxy)benzylidene]-thiazolidine-2,4-dione (1)

Obtained by recrystallization as a yellow solid (1.21 g, 88% yield); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.19 (s, 1H), 7.73 (s, 1H), 7.56 (d, J = 8.7 Hz, 2H), 7.11 (d, J = 8.7 Hz, 2H), 4.17 (t, J = 10.8 Hz, 2H), 3.09 (t, J = 10.2 Hz, 4H), 3.03 (t, J = 10.2 Hz, 4H), 2.95 (t, J = 10.8 Hz, 2H).

3.4.2. 5-(4-(2-(Thiophen-2-yl)ethoxy)benzylidene)thiazolidine-2,4-dione (2)

Obtained by recrystallization as a yellow solid (1.59 g, 87.8% yield); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.12 (s, 1H), 7.73 (s, 1H), 7.56 (d, J = 8.7 Hz, 2H), 7.35 (d, J = 6.0 Hz, 1H), 7.12 (d, J = 8.7 Hz, 2H), 6.94–6.97 (m, 2H), 4.28 (t, J = 12.6 Hz, 2H), 3.285 (t, J = 12.6 Hz, 2H).

3.4.3. 5-(4-(2-Morpholinoethoxy)benzylidene)thiazolidine-2,4-dione (3)

Obtained by recrystallization as a yellow solid (1.16 g, 81.7% yield); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.14 (s, 1H), 7.71 (s, 1H), 7.54 (d, J = 8.7 Hz, 2H), 7.10 (d, J = 8.7 Hz, 2H), 4.22 (t, J = 11.4 Hz, 2H), 3.81 (t, J = 9.6 Hz, 4H), 3.13 (t, J = 11.4 Hz, 2H), 2.76 (t, J = 9.6 Hz, 4H).

3.4.4. 5-(4-(2-(Thiophen-3-yl)ethoxy)benzylidene)thiazolidine-2,4-dione (4)

Obtained by recrystallization as a yellow solid (1.24 g, 86% yield); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.12 (s, 1H), 7.69 (s, 1H), 7.56 (d, J = 11.7 Hz, 2H), 7.45 (d, J = 7.8 Hz, 1H), 7.31 (d, J = 7.8 Hz, 1H), 7.11 (d, J = 11.7 Hz, 2H), 7.09 (s, 1H), 4.28 (t, J = 13.8 Hz, 2H), 3.07 (t, J = 13.8 Hz, 2H).

3.4.5. 5-(4-(2-Isopropoxyethoxy)benzylidene)thiazolidine-2,4-dione (5)

Obtained by recrystallization as a yellow solid (1.26 g, 85.7% yield); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.04 (s, 1H), 7.81 (s, 1H), 7.43 (d, J = 9.0 Hz, 2H), 7.02 (d, J = 9.0 Hz, 2H), 4.19 (t, J = 9.6 Hz, 2H), 3.84 (t, J = 9.6 Hz, 2H), 3.65–3.76 (m, 1H), 1.24 (d, J = 6.0 Hz, 6H).

3.4.6. 5-(4-(2-(Pyridin-2-yl)ethoxy)benzylidene)thiazolidine-2,4-dione (6)

Obtained by recrystallization as a yellow solid (1.21 g, 84.6% yield); ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.36 (s, 1H), 8.45 (d, J = 4.8 Hz, 1H), 7.78 (s, 1H), 7.719–7.28 (m, 1H), 7.49 (d, J = 8.4 Hz, 2H), 7.28 (d, J = 7.8 Hz, 1H), 7.19–7.23 (m, 1H), 6.92 (d, J = 8.4 Hz, 2H), 4.17 (t, J = 14.7 Hz, 2H), 3.05 (t, J = 14.7 Hz, 2H).

3.4.7. 5-(4-(2-(Cyclohexylamino)ethoxy)benzylidene) thiazolidine-2,4-dione (7)

Obtained by recrystallization as a yellow solid (1.11 g, 75.5% yield); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.50 (d, J = 8.7 Hz, 2H), 7.31 (s, 1H), 7.07 (d, J = 8.7 Hz, 2H), 4.24 (t, J = 9.9 Hz, 2H), 3.30 (t, J = 9.9 Hz, 2H), 2.88–2.94 (m, 1H), 2.28 (s, 1H), 1.894–2.07 (m, 2H), 1.733–1.89 (m, 2H), 1.57–1.61 (m, 1H), 1.09–1.30 (m, 4H).

3.4.8. 5-(4-(2-(Tetrahydro-2*H*-pyran-2-yl)ethoxy)benzylidene)-thiazolidine-2,4-dione (8)

Obtained by recrystallization as a yellow solid (1.27 g, 88.2% yield); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.29 (s, 1H), 7.73 (s, 1H), 7.55 (d, J = 9.0 Hz, 2H), 7.10 (d, J = 9.0 Hz, 2H), 3.98 (d, J = 5.1 Hz, 2H), 3.85–3.89 (m, 2H), 3.60–3.64 (m, 1H), 1.97–2.12 (m, 4H), 1.60–1.86 (m, 2H), 1.28–1.47 (m, 2H).

3.4.9. 5-(4-(2-(Piperidin-1-yl)ethoxy)benzylidene)thiazolidine-2,4-dione (9)

Obtained by recrystallization as a yellow solid (1.13 g, 79.6% yield); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.52 (s, 1H), 7.41 (d, J = 10.2 Hz, 2H), 6.97 (d, J = 10.2 Hz, 2H), 4.10 (t, J = 11.1 Hz, 2H), 2.81 (t, J = 11.1 Hz, 2H), 2.38 (m, 4H), 1.41–1.51 (m, 4H), 1.29–1.31 (m, 2H).

3.4.10. 5-(4-(2-(4-Methylthiazol-5-yl)ethoxy)benzylidene) thiazolidine-2,4-dione (10)

Obtained by recrystallization as a yellow solid (1.27 g, 88% yield); ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.49 (s, 1H), 8.82 (s, 1H), 7.72 (s, 1H), 7.56 (d, J = 8.7 Hz, 2H), 7.10 (d, J = 8.7 Hz, 1H), 4.24 (t, J = 12.3 Hz, 2H), 3.25 (t, J = 12.3 Hz, 2H), 2.28 (s, 3H).

3.4.11. 5-[4-(3-Thiomorpholine-1,1-dioxidepropoxy) benzylidene]-thiazolidine-2,4-dione (11)

Obtained by recrystallization as a yellow solid (1.07 g, 81.1%yield); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.19 (s, 1H), 7.71 (s, 1H), 7.54 (d, J = 8.1 Hz, 2H), 7.09 (d, J = 8.1 Hz, 2H), 4.09 (t, J = 12 Hz, 2H), 3.06–3.89 (m, 8H), 2.62 (t, J = 14.1 Hz, 2H), 1.82–1.91 (m, 2H).

3.4.12. 5-(4-(Thiophen-2-ylmethoxy)benzylidene)thiazolidine-2,4-dione (12)

Obtained by recrystallization as a yellow solid (1.17 g, 80.7% yield); ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.51 (s, 1H), 7.75 (s, 1H), 7.57 (d, J = 8.4 Hz, 2H), 7.56 (t, J = 2.4 Hz, 1H), 7.25 (t, J = 3.3 Hz, 1H), 7.20 (d, J = 8.4 Hz, 2H), 7.05 (m, 1H), 5.37 (s, 2H).

3.4.13. 5-(4-(Thiophen-3-ylmethoxy)benzylidene)thiazolidine-2,4-dione (13)

Obtained by recrystallization as a yellow solid (1.24 g, 85.5% yield); ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.50 (s, 1H), 8.59 (s, 1H), 7.86 (d, J = 7.8 Hz, 1H), 7.73 (s, 1H), 7.57 (d, J = 8.7 Hz, 2H), 7.52 (d, J = 7.8 Hz, 1H), 7.20 (d, J = 8.7 Hz, 2H), 5.25 (s, 2H).

3.4.14. 5-(4-(2-Cyclopentylethoxy)benzylidene)thiazolidine-2,4-dione (14)

Obtained by recrystallization as a yellow solid (1.16 g, 81% yield); ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.50 (s, 1H), 7.74 (s, 1H), 7.55 (d, J = 8.7 Hz, 2H), 7.09 (d, J = 8.7 Hz, 2H), 4.14 (t, J = 13.2 Hz, 2H), 1.81–1.98 (m, 1H), 1.700–1.77 (m, 4H), 1.46–1.61 (m, 4H), 1.10-1.19 (m, 2H).

3.4.15. 5-(4-(Furan-2-ylmethoxy)benzylidene)thiazolidine-2,4-dione (15)

Obtained by recrystallization as a yellow solid (1.26 g, 84.6% yield); ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.52 (s, 1H), 7.75 (s, 1H), 7.71 (t, J = 1.8 Hz, 1H), 7.57 (d, J = 8.4 Hz, 1H), 7.20 (d, J = 8.4 Hz, 2H), 6.63 (d, J = 3.0 Hz, 1H), 6.48 (d, J = 1.8 Hz, 1H), 5.14 (s, 2H).

3.4.16. 5-(4-(Pyridin-2-ylmethoxy)benzylidene)thiazolidine-2,4-dione (16)

Obtained by recrystallization as a yellow solid (1.24 g, 84.9% yield); ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.53 (s, 1H), 8.59 (d, J = 4.2 Hz, 1H), 7.87 (t, J = 16.8 Hz, 1H), 7.74 (s, 1H), 7.58 (d, J = 9.0 Hz, 2H), 7.53 (d, J = 7.8 Hz, 1H), 7.37 (m, 1H), 7.20 (d, J = 9.0 Hz, 2H), 5.25 (s, 2H).

3.4.17. 5-(4-(4-Methoxybenzyloxy)benzylidene)thiazolidine-2,4-dione (17)

Obtained by recrystallization as a yellow solid (1.19 g, 85.9% yield); ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.52 (s, 1H), 7.90 (s, 1H) 7.47 (d, J = 8.7 Hz, 2H), 7.25 (d, J = 8.7 Hz, 2H), 7.31 (d, J = 8.4 Hz, 2H), 7.17 (d, J = 8.4 Hz, 2H), 5.06 (s, 2H), 3,79 (s, 3H).

3.4.18. 5-(4-(4-Methylbenzyloxy)benzylidene)thiazolidine-2,4-dione (18)

Obtained by recrystallization as a yellow solid (1.15 g, 80.4% yield); ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.51 (s, 1H), 7.80 (s, 1H), 7.56 (d, J = 8.7 Hz, 2H), 7.35 (d, J = 7.8 Hz, 2H), 7.21 (d, J = 7.8 Hz, 2H), 7.17 (d, J = 8.7 Hz, 2H), 5.13 (s, 2H), 2.30 (s, 3H).

3.4.19. 5-(4-(Benzo[*d*][1,3]dioxol-5-ylmethoxy) benzylidene)-thiazolidine-2,4-dione (19)

Obtained by recrystallization as a yellow solid (1.22 g, 88.4% yield); ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.50 (s, 1H), 7.72 (s, 1H), 7.56 (d, J = 9.0 Hz, 2H), 7.16 (d, J = 9.0 Hz, 2H), 7.01 (s, 1H), 6.96 (d, J = 8.1 Hz, 2H), 6.92 (d, J = 8.1 Hz, 1H), 6.01 (s, 2H), 5.06 (s, 2H).

3.4.20. 5-(4-(Cyclopentylmethoxy)benzylidene)thiazolidine-2,4-dione (20)

Obtained by recrystallization as a yellow solid (1.16 g, 78.0% yield); ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.50 (s, 1H), 7.73 (s, 1H), 7.55 (d, J = 9.0 Hz, 2H), 7.09 (d, J = 9.0 Hz, 2H), 3.92 (d, J = 7.2 Hz, 2H), 2.25–2.35 (m, 1H), 1.75–1.77 (m, 2H), 1.53–1.60 (m, 4H), 1.28–1.34 (m, 2H).

3.4.21. 5-(4-(4-(Chloromethyl)benzyloxy)benzylidene) thiazolidine-2,4-dione (21)

Obtained by recrystallization as a yellow solid (1.12 g, 81.2% yield); ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.55 (s, 1H), 7.75 (s, 1H), 7.58 (d, J = 12.3 Hz, 2H), 7.45 (m, 4H), 7.19 (d, J = 12.3 Hz, 2H), 5.22 (s, 2H), 4.76 (s, 2H).

3.4.22. 5-(4-((4-Methylcyclohexyl)methoxy)benzylidene) thiazolidine-2,4-dione (22)

Obtained by recrystallization as a yellow solid (1.17 g, 85.6% yield); ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.49 (s, 1H), 7.69 (s, 1H), 7.54 (d, J = 9.0 Hz, 2H), 7.10 (d, J = 9.0 Hz, 2H), 3.96 (d, J = 6.9 Hz, 1H), 3.85 (d, J = 6.6 Hz, 1H), 1.66–1.98 (m, 4H), 1.19–1.52 (m, 4H), 0.98–1.16 (m, 2H), 0.85–0.94 (m, 3H).

3.4.23. 5-(4-(2-(Cyclohexyloxy)ethoxy)benzylidene) thiazolidine-2,4-dione (23)

Obtained by recrystallization as a yellow solid (1.14 g, 82.0% yield); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.65 (s, 1H), 7.73 (s, 1H), 7.44 (d, J = 14.7 Hz, 2H), 7.02 (d, J = 14.7 Hz, 2H), 4.20 (t, J = 9.9 Hz, 2H), 3.30 (t, J = 9.9 Hz, 2H), 3.32–3.40 (m, 1H), 1.96–2.18 (m, 2H), 1.75–1.77 (m, 2H), 1.55–1.59 (m, 1H), 1.22–1.39 (m, 5H).

3.4.24. 5-(4-((2,3-Dihydrobenzo[b][1,4]dioxin-2-yl)methoxy)benzylidene)thiazolidine-2,4-dione (24)

Obtained by recrystallization as a yellow solid (1.12 g, 82.4% yield); ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.51 (s, 1H), 7.73 (s, 1H), 7.58 (d, J = 8.7 Hz, 2H), 7.17 (d, J = 8.7 Hz, 2H), 6.82–6.92 (m, 4H), 4.46–4.58 (m, 1H), 4.41–4.46 (m, 1H), 4.26–4.41 (m, 2H), 4.11–4.17 (m, 1H).

3.4.25. Methyl 4-((4-((2,4-dioxothiazolidine-5-ylidene)methyl)-phenoxy)methyl)cyclohexanecarboxylate (25)

Obtained by recrystallization as a yellow solid (1.03 g, 74.1% yield); ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.48 (s, 1H), 7.74 (s, 1H), 7.55 (d, J = 15.9 Hz, 4H), 7.58 (t, J = 9.0 Hz, 2H), 7.10 (d, J = 9.0 Hz, 2H), 3.90 (d, J = 6.6 Hz, 2H), 3.61 (s, 3H), 2.60–2.62 (m, 1H), 1.89–1.98 (m, 3H), 1.45–1.68 (m, 4H), 1.27–1.34 (m, 2H).

3.4.26. 5-(4-(Biphenyl-4-ylmethoxy)benzylidene)thiazolidine-2,4-dione (26)

Obtained by recrystallization as a yellow solid (1.12 g, 83.6% yield); ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.51 (s, 1H), 7.74 (s, 1H),

7.70 (t, J = 15.9 Hz, 4H), 7.58 (t, J = 15.9 Hz, 4H), 7.49 (t, J = 14.7 Hz, 2H), 7.38 (m, 1H), 7.21 (d, J = 8.7 Hz, 2H), 5.24 (s, 2H).

3.4.27. 5-(4-(2-Cyclohexylethoxy)benzylidene)imidazolidine-2,4-dione (27)

Obtained by recrystallization as a yellow solid (1.19 g, 83.0% yield); ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.21 (s, 1H), 10.37 (s, 1H), 7.52 (d, J = 7.8 Hz, 2H), 6.88 (d, J = 7.8 Hz, 2H), 6.32 (s, 1H), 3.94 (t, J = 12.6 Hz, 2H), 1.66 (t, J = 12.6 Hz, 2H), 1.58–1.92 (m, 4H), 1.03–1.27 (m, 5H), 0.79–0.86 (s, 2H).

3.4.28. 3-(4-(2-Cyclohexylethoxy)benzylidene)pyrrolidine-2,5-dione (28)

Obtained by recrystallization as a yellow solid (1.2 g, 84% yield); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.31 (s, 1H), 7.30 (s, 1H), 7.06 (d, J = 11.7 Hz, 2H), 6.84 (d, J = 11.7 Hz, 2H), 3.99 (t, J = 13.2 Hz, 2H), 3.85 (s, 2H), 1.63 (t, J = 13.2 Hz, 2H), 1.52–1.77 (m, 6H), 1.43–1.51 (m, 1H), 1.13–1.27 (m, 2H), 0.90–1.01 (m, 2H).

3.5. General procedure for the synthesis of compounds 29–30

Sodium hydride (21.24 mg, 0.885 mmol, 60% dispersion in oil) was added to a solution of **TD-8** (160 mg, 0.48 mmol) in THF (20 mL) at 0 °C over a 10 min period with constant stirring under nitrogen. The mixture was stirred for an additional 10 min. A solution of iodomethane (205.54 mg, 1.45 mmol) in THF (5 mL) was added slowly to the reaction mixture and stirred at room temperature for 3 h. The reaction mixture was then extracted with ethyl acetate and washed with water. The organic layer was dried over anhyd magnesium sulfate and evaporated. The residual oil was purified by chromatography over silica gel (elution with hexane/ethyl acetate, 10:1) to afford compound **29** (142 mg, 85%) as a white solid.

3.5.1. 5-(4-(2-Cyclohexylethoxy)benzylidene)-3-methylthiazolidine-2,4-dione (29)

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.87 (s, 1H), 7.48 (d, J = 8.7 Hz, 2H), 6.99 (d, J = 8.7 Hz, 2H), 4.08 (t, J = 13.2 Hz, 2H), 3.24 (s, 3H), 1.67–1.78 (m, 4H), 1.45–1.54 (m, 1H), 1.11–1.26 (m, 4H), 0.83–1.05 (m, 4H).

3.5.2. 5-(4-(2-Cyclohexylethoxy)benzylidene)-3-(2-hydroxy ethyl)-thiazolidine-2,4-dione (30)

Obtained by chromatography over silica gel (elution with hexane/ethyl acetate, 10:1) as a white solid (180 mg, 79% yield); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.88 (s, 1H), 7.49 (d, J = 14.4 Hz, 2H), 7.01 (d, J = 14.4 Hz, 2H), 4.08 (t, J = 13.2 Hz, 2H), 4.00 (t, J = 10.2 Hz, 2H), 3.89 (t, J = 10.2 Hz, 2H), 2.05 (m, 1H), 1.67–1.78 (m, 7H), 1.47–1.53 (m, 1H), 1.18–1.28 (m, 3H), 0.96–1.03 (m, 2H).

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