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Original article

Structural exploration, synthesis and pharmacological evaluation of novel 5-benzylidenethiazolidine-2,4-dione derivatives as iNOS inhibitors against inflammatory diseases

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ABSTRACT

In our previous work, **3I** inhibited the LPS-induced iNOS activity and NO production in RAW 264.7 cells and improved joint inflammation and cartilage destruction in inflammatory model. In this study, we synthesized **59** derivatives and bioisosteres on the basis of **3I** by Knoevenagel condensation and biologically evaluated for the study of structure-activity relationship (SAR). We found that **7**–**44** suppressed the iNOS activity (IC_{50} 25.2 μM) and LPS-induced NO production (IC_{50} 45.6 μM) in RAW 264.7 cells. As for the SAR study, the dimethoxylphenyl group of **7**–**44** was potential for a further modification. At a dose of 10 mg/kg, oral administration of **7**–**44** possessed protective properties in both carrageenan-induced paw edema of male ICR mice and adjuvant-induced arthritis of Lewis female rats. Although the activity of **7**–**44** was slightly inferior, the PK profiles of 7–44 were superior to those of **3I**.

1. Introduction

Inflammation is a complex process involving numerous mediators of cellular and plasma origins with interrelated biological functions [1]. Macrophages, neutrophils and lymphocytes play important roles in the pathogenesis of various types of inflammatory diseases such as rheumatoid arthritis, osteoarthritis, inflammatory bowel disease and multiple sclerosis [2]. Proinflammatory stimuli activate cellular responses and regulate inflammatory immune functions including the recruitment of macrophages and the production of cytokines [3].

NO, being an endogenous free radical, is a unique mediator in the process of vasodilation, non-specific host defense and acute or chronic inflammation [4,5]. NO is generated via the oxidation of L-arginine by nitric oxide synthase (NOS), with the consumption of molecular oxygen, NADPH and other cofactors [6]. In mammals, there are at least three isoforms of the NOS enzymes: endothelial, neuronal and inducible [7]. The constitutive neuronal NOS (nNOS) and endothelial NOS (eNOS) are Ca^{2+}-dependent enzymes that play key roles in the nervous and cardiovascular systems [8]. The expression of inducible NOS (iNOS) could be activated by the stimulation of cytokines (e.g., TNF-α) or bacterial lipopolysaccharide (LPS) and further induces large quantities of cytotoxic NO following immunological challenge in macrophages or other cells [9,10]. Considerable evidences revealed that suppression of overproduction of iNOS-mediated NO might be useful drug target for the treatment of inflammatory diseases [11,12].

In our previous work, we developed that the IC_{50} values of (Z)-N-(3-chlorophenyl)-2-(4-((2, 4-dioxothiazolidin-5-ylidene) methyl)phenoxy)acetamide **3I** (**SKLB023**) on LPS-induced iNOS activity and NO production in RAW 264.7 macrophages were 8.66 and 23.55 respectively [13]. Our results exhibited that **3I** significantly improved joint inflammation and cartilage destruction in three inflammatory models of carrageenan-induced paw edema, adjuvant-induced arthritis (AIA) and collagen-induced arthritis

Abbreviations: NOS, nitric oxide synthase; NO, nitric oxide; LPS, lipopolysaccharide; NF-κB, nuclear factor-κB; TNF-α, tumor necrosis factor-α; IL-1β, interleukin-1β; AIA, adjuvant-induced arthritis.

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(CIA) [14]. Furthermore, **3I** inhibited NF-κB activity in peritoneal macrophages by luciferase assay and the number of macrophages in synovial tissues. The levels of NO, TNF-α and IL-1β in plasma and peritoneal macrophages and the expression of iNOS *in vivo* were down-regulated by **3I** at the different doses. Additionally, **3I** suppressed the phosphorylation of components of the mitogen-activated protein kinases (MAPKs) including p38, ERK1/2 and JNK by western blotting [15]. However, the oral bioavailability (F%) of **3I** is only 18.1% which is not suitable as a drug-like candidate [13]. In view of above-mentioned advantages and disadvantages of anti-inflammatory agent 3I, these encourage us to explore novel derivatives or analogues to inhibit the iNOS-mediated NO production for the treatment of inflammatory diseases.

There were two purposes to perform the structural explorations on the basis of lead compound **3I** in the study: to obtain the more potent anti-inflammatory agents and/or to get more drug-like compounds with the comparable anti-inflammatory activity. So, by the combination of various chemical methods, fifty-nine **3I** derivatives and bioisosteres in the present study have been successfully synthesized. Numerous chemical substituents or groups (Fig. 1, such as Saturated or unsaturated aliphatic chain, Polar or hydrophilic aliphatic chain, Substituted aromatic ring, and Bioisosterism by the replacement of other heterocycles, etc.) have been introduced to these derivatives and bioisosteres for the improvement in activity and/or druggability. Furthermore, these compounds were evaluated for the inhibition of LPS-induced NO production in RAW 264.7 macrophages and the treatment of carrageenan-induced paw edema in ICR mice and adjuvant-induced arthritis in Lewis female rats.

2. Chemistry

The intermediates **2** were obtained by the combination of 3-chloroaniline (**1**) with chloroalkynoyl chloride in the presence of triethylamine (Scheme 1S in Supplementary Material). The **2** were further converted into iodoalkylamides **3** by NaI. The **4** were accomplished by employing hydroxybenzaldehydes, **3**, and K_2CO_3 [15]. In Scheme 1, the intermediates **6** was prepared through the condensation of appropriate halides (R_2-X) with thiazolidine-2,4-dione in three different procedures: (**1**) method **A** employed NaH as base and DMF as solvent; (**2**) method **B** used anhydrous K_2CO_3 as base and acetone as a valid solvent; (**3**) method **C** applied Cs_2CO_3 as base and CH_3CN as solvent. Derivatives from 7-**1** to 7-**48** (Scheme 1) were prepared in good yields via a Knoevenagel condensation between the corresponding hydroxybenzaldehydes **4** and a range of substituted thiazolidine- 2,4-dione cores **6** [16,17]. Three conditions have been developed for the Knoevenagel condensation: (**4**) method **D** was performed in the presence of piperidine (cat.) and EtOH solvent; (**5**) method **E** was refluxed by the application of glacial acetic acid and β-alanine; (**6**) method **F** was heated to reflux in the mixture of piperidine (cat.), glacial acetic acid (cat.) and toluene as solvent.

The preparation of compound **7-49** has been obtained in a tandem six-step sequence from 3-chloroaniline (**1**) as described in Scheme 2. Treatment of **1** with BOC-glycine in the presence of EDCI and DMAP (cat.) in ice bath afforded **2-49** with a good yield. The chemical reduction of **2-49** using the $LiAlH_4$ in THF solvent yielded the compound **3-49**. The intermediates **4-49** were obtained by the combination of **3-49** with 2-chloroacetyl chloride in the presence of triethylamine. The aldehyde **5-49** was accomplished by employing **4-49**, 4-hydroxybenzaldehyde, anhydrous K_2CO_3 and KI in refluxed acetone. The Knoevenagel condensation of **5-49** with thiazolidine-2,4-dione to give **6-49** was carried out by employing the piperidine, benzoic acid, and toluene-EtOH (v/v = 1:1) solvent. Finally, **6-49** was deprotected by trifluoroacetic acid (TFA) and further washed by aqueous $NaHCO_3$ to give the product **7-49**.

As for the bioisosteres of thiazolidine-2,4-dione, compound **8a** was synthesized by two-step easy sequence from commercially available pyridin-2-amine as described in Scheme 2S Of Supplementary Material [18]. The **8a** reacted with aldehyde

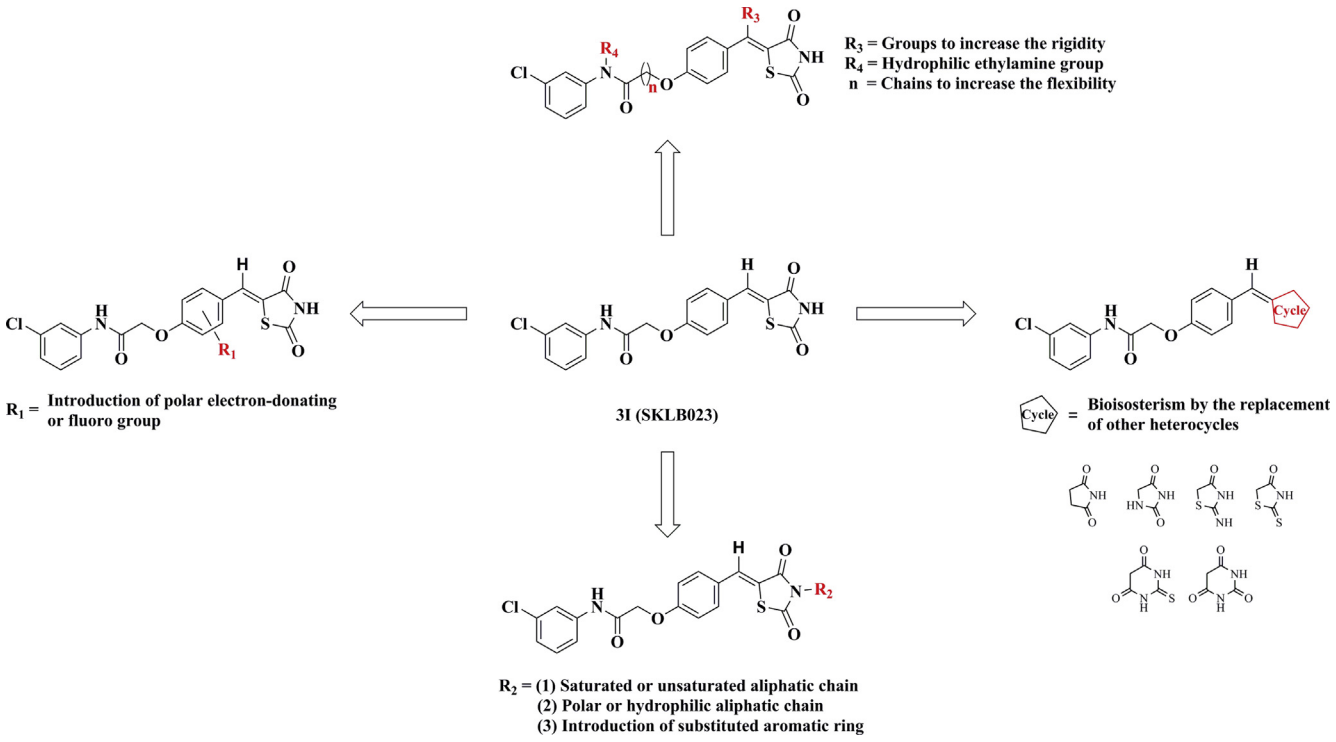
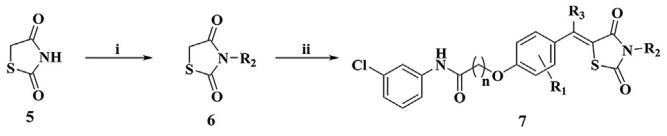
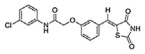


Fig. 1. Structural explorations based on anti-inflammatory agent 3I (SKLB023).



	Compd.	R_2	Compd.	R_2	Compd.	R_2
	7-1	-Et	7-14		7-27	
	7-2		7-15		7-28	
	7-3		7-16		7-29	
	7-4		7-17		7-30	
	7-5		7-18		7-31	
R_1 = H R_3 = H n = 1	7-6		7-19		7-32	
	7-7		7-20		7-33	
	7-8		7-21		7-34	
	7-9		7-22		7-35	
	7-10		7-23		7-36	
	7-11		7-24		7-37	
	7-12		7-25		7-38	
	7-13		7-26		7-39	

Compd.	Structure	Compd.	Structure
7-40		7-45	
7-41		7-46	
7-42		7-47	
7-43		7-48	
7-44			

Scheme 1. Synthesis of **3I**-based derivatives **7** [a]. [a] Reagents and Conditions: (i) Method A: R_2-X, NaH, DMF, 0 °C to r.t, overnight; Method B: R_2-X, K_2CO_3, acetone, reflux, overnight; Method C: R_2-X, Cs_2CO_3, CH_3CN, reflux, overnight; (ii) Method D: **4**, piperidine (cat.), EtOH, reflux, 6 h; Method E: **4**, β-alanine, AcOH, 100 °C; Method F: 4, piperidine (cat.), AcOH (cat.), toluene, reflux, overnight.

intermediate **4a** in NaOAc and AcOH at 100 °C to give the **3I**-based bioisostere **7-50**. The condensation of imidazolidine-2,4-dione derivatives (**8c-e**) with **4a** employing pyrrolidine (cat.) and EtOH solvent afforded **7-51**, **7-52**, and **7-53**, respectively (Scheme 3) [19]. Treatment of 1*H*-pyrrole-2,5-dione with triphenylphosphine (PPh_3) in refluxed MeOH afforded the compound **8b** and further condensed with **4a** via a Witting reaction to give final product **7-54** (Scheme 2S) [20]. Similarly, the Knoevenagel reaction of **4a** with barbituric acid or thiobarbituric acid in a mixture of ethanol/water as valid solvent was refluxed for 5 h to afford the targeted compound **7-55** (X = O), and **7-56** (X = S) [21]. Finally, the commercially available heterocyclic cores of rhodanine, rhodanine-N-acetic acid and 2-iminothiazolidin-4-one were treated with **4a** in the presence of piperidine (cat.) and EtOH to give the bioisosteric derivatives of thiazolidine-2,4-dione **7-57**, **7-58**, and **7-59**, respectively (Scheme 3).

At this stage, all compounds were fully analyzed and characterized by nuclear magnetic resonance (NMR), mass spectrometry and high-performance liquid chromatography (HPLC) before entering the biological screening.

3. Biological results and discussion

3.1. Inhibition and cytotoxicity in RAW 264.7 microphages

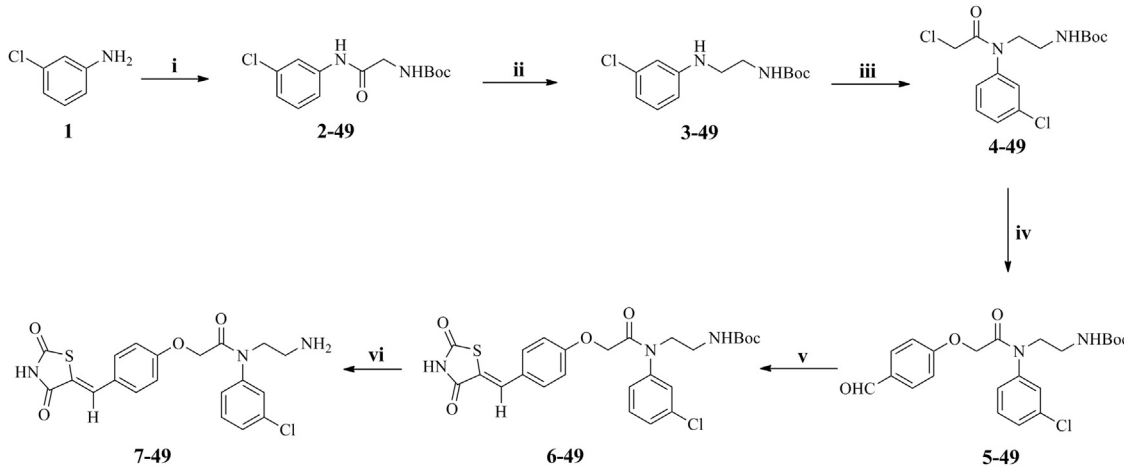
Lipopolysaccharide (LPS) is an important structural component of the outer membrane of gram-negative bacteria, and it is also a well-studied immunostimulator that induced an immune inflammatory response, and especially, the expression of NO production and other proinflammatory cytokines. Here, 59 synthetic **3I** derivatives and bioisosteres were evaluated for inhibitory activity against LPS-induced NO production in RAW 246.7 cells. The macrophages were pre-treated with compounds for 2 h and then incubated with LPS (1 μg/mL) for 18 h. The amounts of NO released into culture media was detected according to the *Griess* method in the form of nitrite. Here, **3I** and *indomethacin* were chosen as a positive control.

As depicted in Fig. 1, we found that 12 compounds (**7-5**, **7-10**, **7-14**, **7-16**, **7-17**, **7-32**, **7-40**, **7-44**, **7-52**, **7-56**, **7-58**, and **7-59**) partially suppressed NO production at a concentration of 100 μM and **7-44** was the more comparable anti-inflammatory effect among fifty-nine synthetic derivatives and bioisosteres. The positive **3I** and *Indomethacin* exhibited the most potent inhibitory activities. Compared to the most potent **3I**, the introduction of two methoxyl groups into the phenyl ring of 5-benzylidenethiazolidine-2,4-dione moiety led to **7-44**. In addition, the other synthetic derivatives and bioisosteres did not show the significant inhibitory activity.

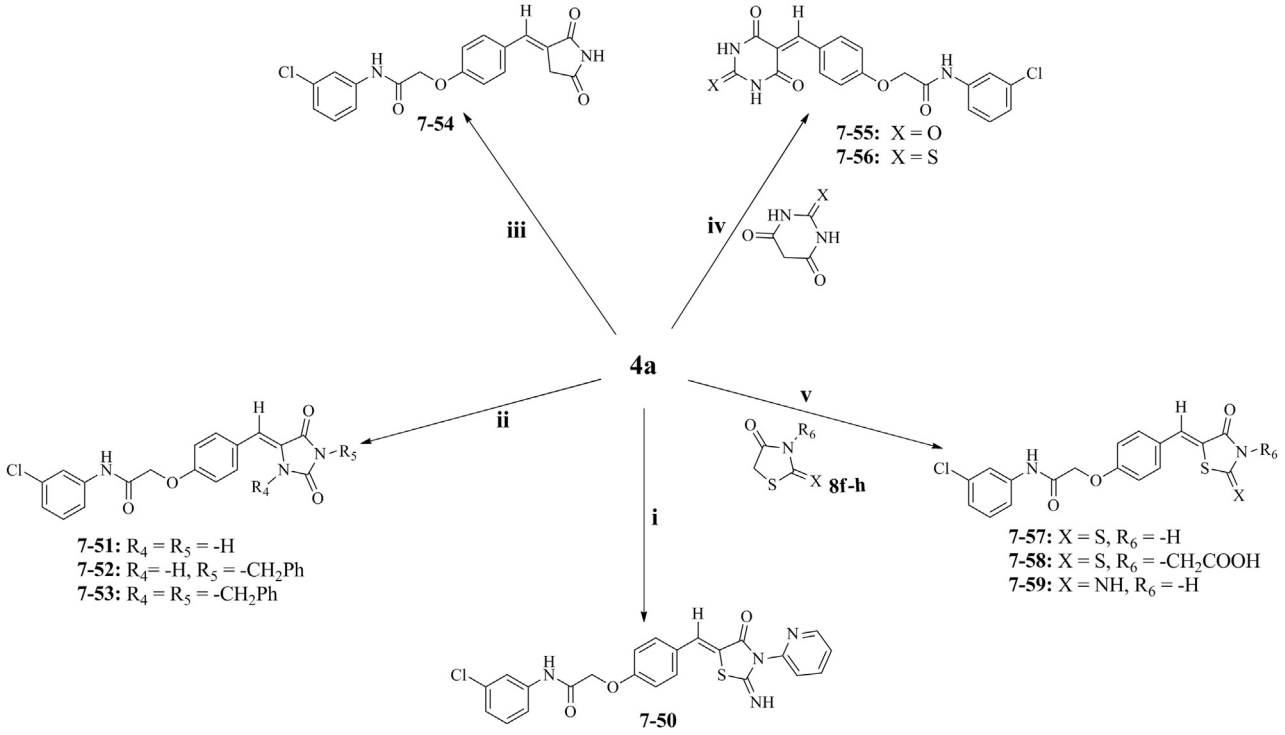
As for the study of structure-activity relationship (SAR), the introduction of chain saturated aliphatic groups (e.g., Ethylated in 7-1, Isobutylated in 7-2 and Butylated in 7-6) and unsaturated (Propenylated in 7-3) into the N-atom site of thiazolidine-2,4-dione failed to improve the inhibitory activity compared to 3I. However, the high unsaturated propynylated derivative (7-5) and cyclo-derivative (7-7, 7-16, and 7-17) exhibited the potential inhibitory potency in contrast to LPS stimulated group. In addition, we could find that the other compounds of N-substituent in thiazolidine-2,4-dione moiety (e.g., polar group acetic acid in 7-8, benzoic acid in 7-31 or big steric effect in 7-35) were non-active to inhibit the NO production without statistical significance in Table 1. Inspection of their structural features in Scheme 1 and their inhibitory activities in Fig. 2 highlighted that the chemical bond of N—H in thiazolidine-2,4-dione was approved to important for the inhibition.

Although aforementioned derivatives exhibited less potent activities against the NO production than that of 3I and *Indomethacin*, our synthetic strategy was further devoted to keep the thiazolidine-2,4-dione moiety intact (Table 2). With respect to these 8 compounds from 7-41 to 7-46, the substituents such as monomethoxyl (7-41 and 7-42), monoethoxyl (7-43), dimethoxyl (7-44) and fluoro (7-45) group of phenyl ring of 5-benzylidenethiazolidine-2,4-dione moiety were explored. We also change the linker site in compound 7-40 or lengthened the linker in compounds 7-47 and 7-48. We found that 7-40 ($P < 0.05$) and 7-44 ($P < 0.01$) were the more efficient, while the 7-44 was more potent that 7-40. Between 7-40 and 7-44, we could find that the *para*-orientation is superior to *meta*-substitution and the number of electron-donating group (EDG) contributed to the inhibition from 7-41 to 7-45. The dimethoxylphenyl ring of 7-44 was worth to be a further structural modification by the introduction of EDG. The inhibitory activity of 7-44 was comparable to that of 3I and positive control *Indomethacin* against the production of NO at the same concentration.

As we all known, the bioisosteric replacement was important



Scheme 2. Synthesis of **3l**-based compound **7-49**[a]. [a] Reagents and Conditions: (i) N-Boc-Gly, EDCI, DMAP (cat.), CH_2Cl_2, 0 °C to r.t, overnight; (ii) $LiAlH_4$, THF, 0 °C to r.t, overnight; (iii) 2-chloroacetyl chloride, $NaHCO_3$, CH_2Cl_2, 0 °C to r.t; overnight; (iv) KI, K_2CO_3, 4-hydroxybenzaldehyde, acetone, reflux, overnight; (v) thiazolidine-2,4-dione, piperidine, benzoic acid, toluene-EtOH (v/v = 1:1), reflux, overnight; (vi) TFA/CH_2Cl_2, 0 °C to r.t, 4 h, then washed by $NaHCO_3$.



Scheme 3. Synthesis of bioisosteric derivatives of **3l**[a]. [a]Reagents and Conditions: (i) **8a**, NaOAc, AcOH, 100 °C, 5 h; (ii) **8c-e**, pyrrolidine (cat.), EtOH, reflux, 5–15 h; (iii) **8b**, MeOH, reflux, overnight; (iv) EtOH–H_2O, reflux, 5 h; (v) piperidine (cat.), EtOH, reflux, 6 h.

theory in structural modification of drug development. There we have synthesized 10 compounds to evaluate the bioisosteres of thiazolidine-2,4-dione (2-Iminothiazolidin-4-ones, Imidazolidine-2,4-diones, Pyrrolidine-2,5-dione, Barbituric acid, Thiobarbituric acid, and 2-Thioxothiazolidin-4-ones from 7-50 to 7-59 in Scheme 2S). Although the four compounds 7-52, 7-56, 7-58 and 7-59 showed the weak inhibition compared to that of 7-44, the bioisosteric replacements was dispensable for the activity to some content and the thiazolidine-2,4-dione moiety still is the best choice. Consequently, the dimethoxylphenyl group of **7-44** was potential for a further modification.

The iNOS mediated the NO production and so selected compounds were further exposed to RAW 264.7 macrophages to explore their inhibitory effects and cytotoxicity. As shown in Table 1, **7-44** exhibited the most potent inhibitory potencies on iNOS activity (IC_{50} = 25.20 μM), and the generation of LPS-mediated NO (IC_{50} = 45.60 μM), whereas the results were inferior to those of 3I and *Indomethacin* on LPS-induced RAW 264.7 cells. To check whether the suppressive effects of these compounds on iNOS, and NO was related to cell viability, MTT assay was adopted. As exhibited in Table 1, 7-14, 7-44 and 7-58 showed no cytotoxicity on RAW 264.7 macrophages (without or with LPS, IC_{50} > 80.00 μM). Similarly, the positive 3I and *indomethacin* did not show any cytotoxicity in the previus study [13]. However, we found that effects of

Table 1
IC_{50} values of NO, iNOS and cytotoxicity and physicochemical property.

Compd.	NO IC_{50} (μM)	iNOS IC_{50} (μM)	Cytotoxicity IC_{50} (μM)		clog P[c]	tPSA[c]	Solubility (mg/mL)[d]		
			−LPS	+LPS			DMSO	EtOH	PBS
3I	21.32	8.66[b]	>80.00	>80.00	2.44	84.50	34.0	1.5	<0.5
Indo[a]	40.30	23.47[b]	>80.00	>80.00	–	–	–	–	–
7-11	>80.00	–	68.40	72.50	2.79	84.94	–	–	–
7-14	53.20	32.10	>80.00	>80.00	3.26	78.95	–	–	–
7-44	45.60	25.20	>80.00	>80.00	2.18	102.96	78.0	3.1	<0.5
7-52	75.35	43.21	>80.00	46.30	3.16	87.74	–	–	–
7-58	68.90	47.80	>80.00	>80.00	2.48	95.94	–	–	–

[a] Indomethacin, an anti-inflammatory drug.
[b] Data from Reference No.12.
[c] Calculated with ChemBioDraw 12.0.
[d] Detected maximum soluble concentration by nephelometry.

7-11 and 7-52 were more cytotoxic than the other compounds, especially in the LPS-induced macrophages. On the basis of cellular viability and anti-inflammatory activity *in vitro*, the more potent derivative 7-44 were further evaluated in the next experimental process.

In addition, at the temperature of 25 °C, the solubility of 7-44 and **3I** in DMSO, phosphate buffer saline (PBS, pH 7.4) and ethanol solution were respectively 78.0 and 43.0 mg/mL, both less than 0.5 mg/mL, 3.1 and 1.5 mg/mL, by detecting maximum soluble concentration by nephelometry. After the introduction of two methoxyl group into 5-benzylidenethiazolidine-2,4-dione slightly improved the solubility profiles compared to 3I, and one possible reason is that two EDG increase the hydrophily of molecule, which was in accordance with the predicted cLogP and tPSA (A^2) data by the ChemBioDraw Ultra 12.0 software. These profiles may be a right guideline for our further structural modification and exploration for potent and drug-like anti-inflammatory agents.

3.2. Pharmacokinetic profiles

Furthermore, a pharmacokinetic profile of 7-44 was determined in male Sprague–Dawley rats (n = 6) following intravenous and oral administration at 5.0 mg/kg and 20 mg/kg, respectively. After an i.p. injection, the results indicated that 7-44 has a high plasma concentration of 331.5 μg/L and a long $t_{1/2}$ of 7.83 h in this species, while the C_{max} and $t_{1/2}$ were 46.2 μg/L and 5.9 h after oral administration. Oral bioavailability (F %) was estimated to be 27.2% on the basis of the $AUC_{0\text{-}t}$ ratio and drug dose. In our previous work, we found that the oral F% and $t_{1/2}$ were 18.1% and 2.16 h [13]. Introduction of two methoxy groups in 7-44 made it comparable activity and preferable oral bioavailability to those of 3I because that the two methoxy groups maybe not change the integrality of **3I**. And by the various structural explorations, we summarized that the thiazolidine-2,4-dione was very important to activity and the phenyl ring of 5-benzylidenethiazolidine-2,4-dione was further worthy of structural modification to improve the druggability. These profiles may be a right guideline for our further structural modification and explorations.

3.3. Docking study of iNOS

To gain better understanding on the potency of the studied compounds, we proceeded to examine the interaction of all compounds with iNOS protein which 3D structure was gained from Protein Data Bank (PDB ID: 1r35) [22]. Molecule was built with ChemBio3D and optimized at molecular mechanical and semi-empirical level by using Hyperchem software [23]. Conformers of compound were created by the aid of Omega [24] and the up limit of conformer number was set to 2000. Then they were docked to the binding site of iNOS by employing a protein-ligand docking program FRED [25]. Scoring function chemgauss 3 [26] was used for exhaustive searching, solid body optimizing and interaction scoring. The pose with the most favorable score was remained. Compounds **3I**, 7-11, 7-14, 7-44 and 7-52 are among the top structures by computational scoring which almost is consistent with *in vitro* iNOS screening (Supplementary material). As depicted in Fig. 3, the meta-chlorophenyl moiety of 7-44 was buried in a pocket between Phe363, Trp366 and an HEME. The linker between the two phenyl groups was enclosed by a polar cavity consisting of Glu371, Asp376 and Mem349. The thiazolidinedione moiety was surrounded by Asn348, Arg260, Tyr485 and Arg382. In addition, two strong hydrogen bonds are formed between 7-44 and Glu371 and Asn348 and a π-π interaction between the meta-chlorophenyl ring of 7-44 and HEME was also observed. From the docking result, the dimethoxylphenyl ring of 7-44 was worth to be a further structural modification.

3.4. Inhibition of carrageenan-induced paw edema and adjuvant-induced arthritis

To evaluate the *in vivo* anti-inflammatory potency of 7-44, carrageenan-induced paw edema of acute inflammation was employed. The edema is an important parameter of acute inflammation for evaluating compounds with potential anti-inflammatory activity. In the carrageenan-induced paw edema test, the inflammatory response was quantified by increment in paw size (edema) 2 h after carrageenan was injected and the paw edema was observed (Fig. 4A). Oral administration of 7-44 at a dose of 10 mg/kg could suppress carrageenan-induced paw edema. Treatment with 10 mg/kg 7-44 exhibited less inhibitory activity than same dose of *indomethacin* and **3I**. 7-44 inhibited edema formation after edema induction with the inhibitory rate of 26.1% ($p < 0.05$) at the primary endpoint, while **3I** and *indomethacin* showed an inhibitory rate of 37.8% ($p < 0.01$) and 33.5% ($p < 0.05$) of edema development, respectively.

Because of the inhibition of carrageen-induced paw edema, 7-44 has been chosen to further be examined on animal model of adjuvant-induced arthritis (AIA). AIA is a well-established experimental model of rheumatoid arthritis and is often used for testing agents for anti-inflammatory activity. In this model, 90–100% of rats developed arthritis within 14–18 days after adjuvant injection. As displayed in Fig. 4B, rats treated with **7-44** at a dose of 10 mg/kg did not develop severe arthritis, indicating that it exhibited potential immune-modulating activity. Arthritic score was reduced in the therapeutic process.

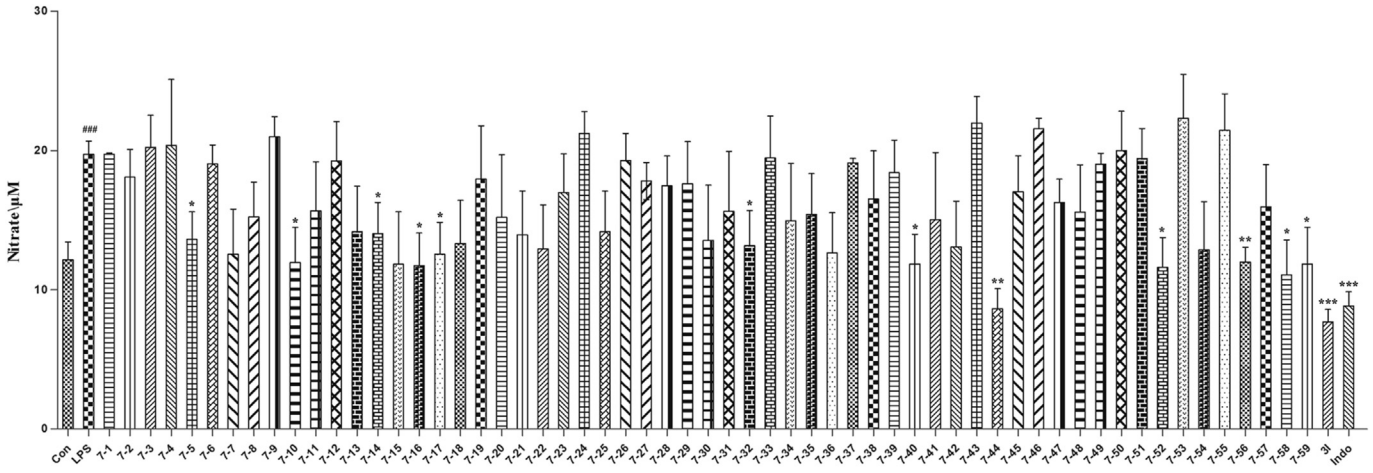


Fig. 2. The amount of nitrate in LPS-induced RAW 264.7 macrophages. Results are means ± SD (n = 3). $^{###}P < 0.001$ *vs.* control. $^{*}P < 0.05$; $^{**}P < 0.01$ and $^{***}P < 0.001$ *vs.* LPS group.

Table 2
Pharmacokinetic Profiles of 7-44 in SD rats.[a]

	ip	op
C_{max} (μg/L)	331.5	46.2
t_{max} (h)	0.07	2.27
$t_{1/2}$ (h)	7.83	5.90
AUC_{0-t} (μg/L h)	387.2	421.1
F (%)		27.2

[a] Data were mean concentrations in mouse plasma (n = 6) following a single 5.0 mg/kg intravenous dose or 20 mg/kg oral dose.

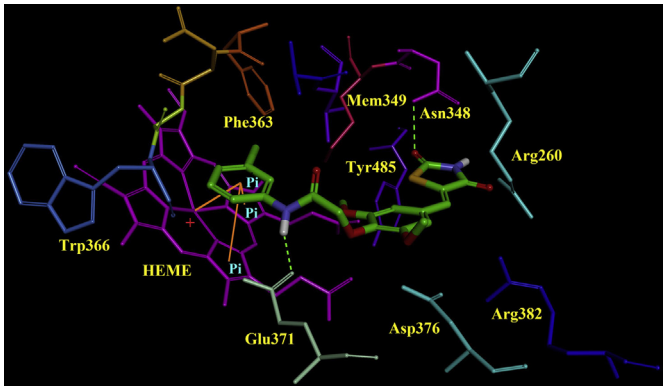


Fig. 3. The interaction mode of **7-44** within the active site of murine iNOS.

4. Conclusion

In this study, we have synthesized 59 derivatives and bioisosteres based on **3I** and evaluated for their inhibitory activity on LPS-induced NO production and iNOS activity. We found that 7-44 suppressed the iNOS activity, and the production of NO in RAW 264.7 cells. Furthermore, treatment of 7-44 at a dose of 10 mg/kg improved the inflammatory response such as decrement of the paw edema and signs of AIA, while the result was a little inferior to that of **3I** at the same dose. The introduction of two methoxyl group into phenyl ring of 5-benzylidenethiazolidine-2,4-dione moiety was slightly inferior to 3I against *in vitro* NO production and iNOS activity. In other side, the limited structural modification improved the PK profiles to add the plasma concentration and make 7-44 exhibit the approximate *in vivo* anti-inflammatory activity to 3I.

5. Experimental section

5.1. Biological methods

5.1.1. Materials

LPS (Escherichia coli serotype 0111:B4), MTT, Carrageenan from seaweed (a mixture of lambda and kappa-carrageenan) and Indomethacin were obtained from Sigma Chemical Co. (St. Louis, MO).

5.1.2. Cell culture

RAW 264.7 macrophages were obtained from ATCC (Rockville, MD, USA). These cells were grown in RPMI 1640 containing 10% FBS, 100 units/mL penicillin, and 100 μg/mL streptomycin in 95% air, 5% CO_2 humidified atmosphere at 37 °C.

5.1.3. Nitrite assay

RAW 264.7 cells were seeded into a 96-well culture plate at a density of 1×10^4 cells per well with 500 μL of culture medium and incubated for 24 h. The cells were then pre-treated with compounds 7 and Indomethacin at 100 μM for 2 h before stimulation with LPS (1 μg/mL) for 18 h. The nitrite concentration in the medium was measured according to Griess reaction by adding 50 μL Griess reagent (1% sulfanilamide and 0.1% N-(1-naphthyl)ethylenediamine dihydrochloride in 5% phosphoric acid) to 50 μL of medium for 5 min. The OD_{540} was measured with a microplate reader. Concentrations were calculated by comparison with OD_{540} of a standard solution of sodium nitrite prepared in culture medium.

5.1.4. Cell cytotoxicity

RAW 264.7 cells were treated with compounds alone or in combination with LPS for 24 h. Cells were washed with PBS and incubated in 0.5 mg/mL MTT reagent dissolved in RPMI 1640 for 4 h, and the formazan product dissolved in 150 μL DMSO. The optical density was measured using an ELISA plate reader at 570 nm (OD_{570}).

5.1.5. Assay of iNOS enzymatic activity

After treated with LPS (1 μg/mL) and **7-44** (1–50 μM) for 2 h at 37 °C, the culture supernatant was removed and 100 μL of NOS assay buffer (1×) were added to each well. Then 100 μL of NOS assay reaction solution (50% NOS assay buffer, 39.8% MilliQ water, 5% L-Arginine solution, 5% 0.1 mM NADPH, 0.2% DAF-FMDA) was added to each well and incubated for 2 h at 37 °C. Fluorescence was

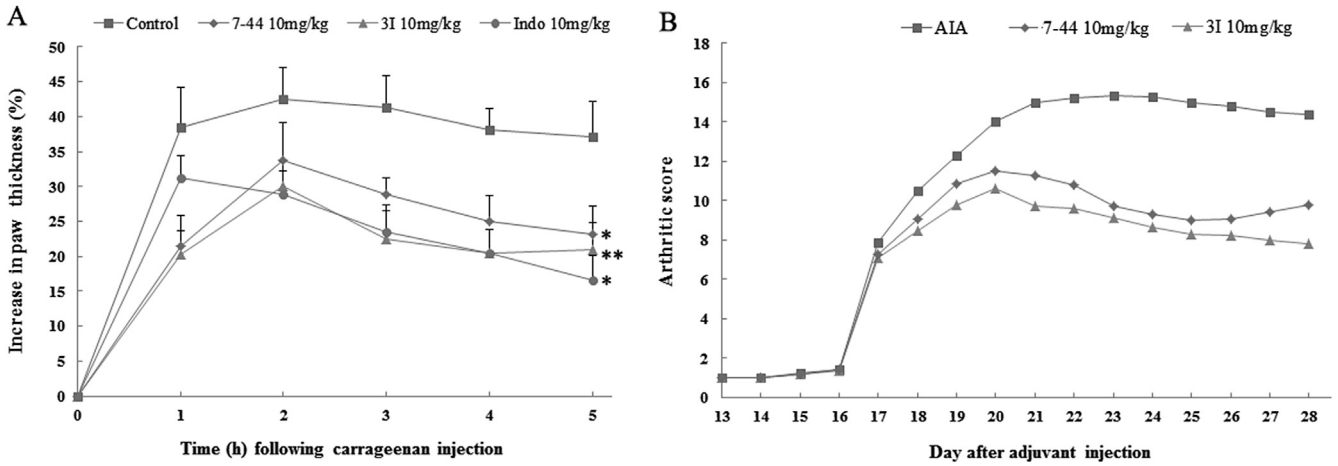


Fig. 4. Inhibition of carrageenan-induced paw edema in ICR mice (A) and adjuvant-induced arthritis in female Lewis rats (B). The results were expressed as the means ± SD (n = 6). $^{*}P < 0.05$; and $^{**}P < 0.01$ *vs.* control.

measured with a fluorescence plate reader (Biotek) at excitation of 485 nm and emission of 528 nm.

5.1.6. Carrageenan-induced paw edema test in ICR mice

ICR male mice and female Lewis rats were housed in filtered-capped polycarbonate cages and allowed food and water *ad libitum*. Animals were kept on a cycle of 12 h light/darkness at 22 ± 1 °C and acclimated for at least one week until use. All protocols of *in vivo* experiments and animals received human cares were operated according to National Institutes of Health Guidelines. The mice were randomly divided into four groups. Doses (10 mg/kg and 50 mg/kg) of compounds were administered i.p. to the test groups, respectively. The positive control group was given access to Indomethacin (10 mg/kg, i.p.) and the vehicle control group was given access to the same volume of olive oil (10 mL/kg, i.p.). Thirty minutes after the administration, acute paw edema was induced in the right hind paw by subplantar injection of 1% freshly prepared carrageenan suspension in normal saline, 50 μL per a mouse. The thickness of the paw was measured pre-injection and at intervals of 1, 2, 3, 4, 5 and 6 h post-injection, using a Dial Thickness Gage. The percent increase of paw thickness was calculated based on the pre-injection thickness of the paw.

5.1.7. Induction and assessment of AIA

Briefly, 12-week-old, gonad-intact, female Lewis rats with six rats per group were injected on the ventral side of the base of the tail with CFA. The degree of arthritis severity was monitored daily and scored according to the following disease indices: hindpaw erythema, hindpaw swelling, tenderness of the joints, and movements, and posture. An integer scale of **0** to **3** is used to quantify the level of erythema (**0**, normal paw; **1**, mild erythema; **2**, moderate erythema; **3**, severe erythema) and swelling (**0**, normal paw; **1**, mild swelling; **2**, moderate swelling; **3**, severe swelling of the hindpaw). The maximal possible score per day is 12, and this score was seen in all rats 8 days after CFA injection. A cohort of Lewis rats not injected with CFA but receiving daily oral doses of vehicle (2% Tween 80/0.5% methylcellulose) for 21 days were designated normal controls. CFA-treated rats were dosed with vehicle for 7 days until full joint inflammation developed and then were randomized into four groups and treated for another 14 days. One group continued to be dosed with vehicle. And the other three groups were treated with **7-44** (10 mg/kg), **3I** (10 mg/kg) and indomethacin (10 mg/kg), respectively.

5.1.8. Statistical analysis

Experimental values are presented as the arithmetic means ± SD. GraphPad Prism 5.0 software followed by unpaired t test with Welch's correction was used to determined statistical differences. $P < 0.05$ was considered to be statistically significant.

5.2. Chemistry

Chemical reagents of analytical grade were purchased from Chengdu Changzheng Chemical Factory (Sichuan, P. R. China). TLC was performed on 0.20 mm silica gel 60 F_{254} plates (Qingdao Ocean Chemical Factory, China). The purity of compounds was determined to be ≥ 97% by HPLC analysis with a photodiode array detector (Waters) and the chromatographic column was a atlantis C_{18} (150 mm × 4.6 mm, i.d. 5 μm) (Waters). ^{1}H NMR was recorded at 400 MHz on a Varian spectrometer model Gemini 400 and reported in parts per million. Chemical shifts (δ) are quoted in ppm relative to TMS as an internal standard, where (δ) TMS = 0.00 ppm. The multiplicity of the signal is indicated as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet, defined as all multipeak signals where overlap or complex coupling of signals makes definitive descriptions of peaks difficult. Mass Spectra were measured by Q-TOF Priemier mass spectrometer utilizing electrospray ionization (ESI) (Micromass).

*5.2.1. General procedure for synthesis of compounds **3***

2-Chloroacetyl chloride (24 mmol) was slowly added to a mixture of R—NH_2 (20 mmol) and Et_3N (24 mmol, 3.3 mL) in anhydrous DCM (20 mL) at 0 °C. The mixture warmed to room temperature and stirred for 20 h. After the solvent was removed, the residue was washed by water (3 × 20 mL) and the precipitate was collected. The crude product **2** was purified by crystallization from ether/petroleum. Then, **2** (1.0 mmol) and NaI (8.0 mmol) were added into acetone (20 mL) and the mixture was refluxed. After the solvent was removed under reduced pressure, the residue **3** was afforded without further purification.

*5.2.2. General procedure for synthesis of compounds **4***

Hydroxybenzaldehyde (11 mmol), K_2CO_3 (2.76 g, 20 mmol) and 4 (10 mmol) were dissolved in acetone (30 mL). The mixture was refluxed overnight and then cooled to room temperature. Then K_2CO_3 solid was filtered and the acetone was removed under reduced pressure to obtain the crude products. The residue was purified by silica gel column chromatography to give the

appropriate aldehydes **4**.

*5.2.3. General procedure for synthesis of compounds **6***

Method A: Thiazolidine-2,4-one (1.0 mmol) was dissolved into DMF (10 mL) at 0 °C and NaH (1.5 mmol, 60%) was slowly added into the solution. R_2-X (1.0 mmol) was added into the mixture and allowed to warm up to room temperature overnight. Then, 25 mL water was added and the mixture was extracted by EtOAc (3 × 15 mL), and the combined organic layer was washed by water (2 × 10 mL) and brine (2 × 10 mL). The solvent was removed to obtain the crude product without purification.

Method B: Thiazolidine-2,4-one (1.0 mmol), R_2-X (1.0 mmol), anhydrous K_2CO_3 (1.5 mmol) were added into acetone (15 mL) and the mixture was refluxed overnight. Then K_2CO_3 solid was filtered, and the solution was collected and further extracted by EtOAc (3 × 15 mL), water (2 × 10 mL), brine (2 × 10 mL), and dried by anhydrous Na_2SO_4. The solvent was removed to obtain the crude product without purification.

Method C: Thiazolidine-2,4-one (1.0 mmol), R_2-X (1.0 mmol), Cs_2CO_3 (1.5 mmol) were added into CH_3CN (15 mL) and the mixture was refluxed overnight. Then K_2CO_3 solid was filtered, and the solution was collected and further extracted by EtOAc (3 × 15 mL), water (2 × 10 mL), brine (2 × 10 mL), and dried by anhydrous Na_2SO_4. The solvent was removed to obtain the crude product without purification.

*5.2.4. General procedure for synthesis of compounds **7***

Method D: The appropriate aldehyde **4** (1.0 mmol), thiazolidine-2,4-one derivatives **6** (1.2 mmol), piperidine (cat.) and 10 mL EtOH were mixed and heated to reflux for 6 h. Then a portion of water/ice was added and the precipitated solids were collected by sucking filtration and washed with distilled water (4 × 15 mL), EtOH (2 × 10 mL), and ether (2 × 10 mL). The solids obtained were dried in vacuum at 40 °C for 24 h.

Method E: The appropriate aldehyde **4** (1.0 mmol), thiazolidine-2,4-one derivatives **6** (2.0 mmol), 10 mL glacial acetic acid, and β-alanine (2.0 mmol) were mixed and heated to reflux for 4 h. Then a portion of water/ice was added and the precipitated solids were collected by sucking filtration and washed with distilled water (4 × 15 mL), EtOH (2 × 10 mL), and ether (2 × 10 mL). The solids obtained were dried in vacuum at 40 °C for 24 h.

Method F: The appropriate aldehyde **4** (1.0 mmol), thiazolidine-2,4-one derivatives **6** (1.2 mmol), piperidine (cat.), glacial acetic acid (cat.) and 10 mL toluene were mixed and heated to reflux overnight. Then a portion of water/ice was added and the precipitated solid was collected by sucking filtration and washed with distilled water (4 × 15 mL), EtOH (2 × 10 mL), and ether (2 × 10 mL). The solids obtained were dried in vacuum at 40 °C for 24 h.

*5.2.4.1. (Z)-N-(3-Chlorophenyl)-2-(4-((3-ethyl-2,4-dioxothiazolidin-5-ylidene)methyl)phenoxy)acetamide **7-1***. Prepared by general procedure for the synthesis of **6** (Method A) and **7** (Method D). Yield 65.3%; White powder; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.34 (s, 1H), 7.89 (s, 1H), 7.83 (t, 1H, J = 2.0 Hz), 7.62 (d, 2H, J = 8.8 Hz), 7.53 (d, 1H, J = 8.0 Hz), 7.36 (t, 1H, J = 8.0 Hz), 7.18–7.14 (m, 3H), 4.83 (s, 2H), 3.67 (q, 2H, J = 7.2 Hz), 1.15 (t, 3H, J = 7.2 Hz); MS (ESI), m/z: 415.05 $[M-H]^-$.

*5.2.4.2. (Z)-N-(3-Chlorophenyl)-2-(4-((3-isopropyl-2,4-dioxothiazolidin-5-ylidene)methyl)phenoxy)acetamide **7-2***. Prepared by general procedure for the synthesis of **6** (Method A) and **7** (Method D). Yield 35.2%; White powder; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.37 (s, 1H), 7.85–7.84 (m, 2H), 7.60 (d, 2H, J = 8.4 Hz), 7.52 (d, 1H, J = 8.0 Hz), 7.36 (t, 1H, J = 8.0 Hz), 7.17–7.15 (m, 3H), 4.83 (s, 2H), 4.53 (qt, 1H, J = 6.8 Hz), 1.40 (s, 3H), 1.38 (s, 3H); MS (ESI), m/z: 429.05 $[M-H]^-$.

*5.2.4.3. (Z)-2-(4-((3-Allyl-2,4-dioxothiazolidin-5-ylidene)methyl)phenoxy)-N-(3-chlorophenyl)acetamide **7-3***. Prepared by general procedure for the synthesis of **6** (Method A) and **7** (Method D). Yield 49.0%; White powder; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.37 (s, 1H), 7.92 (s, 1H), 7.84 (t, 1H, J = 1.6 Hz), 7.64 (d, 2H, J = 8.8 Hz), 7.53 (d, 1H, J = 8.0 Hz), 7.37 (t, 1H, J = 8.0 Hz), 7.18–7.15 (m, 3H), 5.89–5.82 (m, 1H), 5.18–5.13 (m, 2H), 4.84 (s, 2H), 4.26 (d, 2H, J = 7.2 Hz); MS (ESI), m/z: 427.04 $[M-H]^-$.

*5.2.4.4. (Z)-N-(3-Chlorophenyl)-2-(4-((3-(3-methylbut-2-en-1-yl)-2,4-dioxothiazolidin-5-ylidene)methyl)phenoxy)acetamide **7-4***. Prepared by general procedure for the synthesis of **6** (Method A) and **7** (Method D). Yield 54.1%; White powder; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.36 (s, 1H), 7.89 (s, 1H), 7.84 (s, 1H), 7.61 (d, 2H, J = 8.8 Hz), 7.53 (d, 1H, J = 8.8 Hz), 7.36 (t, 1H, J = 8.0 Hz), 7.17–7.14 (m, 3H), 5.18 (m, 1H), 4.83 (s, 2H), 4.22 (d, 2H, J = 6.8 Hz), 1.74 (s, 3H), 1.68 (s, 3H); MS (ESI), m/z: 455.01 $[M-H]^-$.

*5.2.4.5. (Z)-N-(3-Chlorophenyl)-2-(4-((2,4-dioxo-3-(prop-2-yn-1-yl)thiazolidin-5-ylidene)methyl)phenoxy)acetamide **7–5***. Prepared by general procedure for the synthesis of **6** (Method A) and **7** (Method D). Yield 23.1%; White powder; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.37 (s, 1H), 7.96 (s, 1H), 7.84 (t, 1H, J = 2.0 Hz), 7.64 (d, 2H, J = 8.8 Hz), 7.52 (d, 1H, J = 8.4 Hz), 7.37 (t, 1H, J = 8.0 Hz), 7.19–7.14 (m, 4H), 4.84 (s, 2H), 4.23 (d, 2H, J = 2.4 Hz); MS (ESI), m/z: 425.01 $[M-H]^-$.

*5.2.4.6. (Z)-2-(4-((3-Butyl-2,4-dioxothiazolidin-5-ylidene)methyl)phenoxy)-N-(3-chlorophenyl)acetamide **7-6***. Prepared by general procedure for the synthesis of **6** (Method A) and **7** (Method D). Yield 78.0%; White powder; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.36 (s, 1H), 7.93 (s, 1H), 7.84 (s, 1H), 7.62 (d, 2H, J = 8.8 Hz), 7.53 (d, 1H, J = 8.0 Hz), 7.36 (t, 1H, J = 8.0 Hz), 7.17–7.15 (m, 3H), 4.84 (s, 2H), 3.64 (t, 2H, J = 7.2 Hz), 1.54 (q, 2H, J = 7.2 Hz), 1.27 (q, 2H, J = 7.2 Hz), 0.89 (t, 3H, J = 7.2 Hz); MS (ESI), m/z: 443.10 $[M-H]^-$.

*5.2.4.7. (Z)-N-(3-Chlorophenyl)-2-(4-((3-cyclopentyl-2,4-dioxothiazolidin-5-ylidene)methyl)phenoxy)acetamide **7-7***. Prepared by general procedure for the synthesis of **6** (Method A) and **7** (Method D). Yield 87.3%; White powder; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.36 (s, 1H), 7.86–7.84 (m, 2H), 7.60 (d, 2H, J = 8.8 Hz), 7.53 (d, 1H, J = 8.0 Hz), 7.36 (t, 1H, J = 8.4 Hz), 7.17–7.14 (m, 3H), 4.83 (s, 2H), 4.67 (qt, 1H, J = 8.4 Hz), 1.97–1.57 (m, 8H); MS (ESI), m/z: 455.05 $[M-H]^-$.

*5.2.4.8. (Z)-2-(5-(4-(2-((3-Chlorophenyl)amino)-2-oxoethoxy)benzylidene)-2,4-dioxothiazolidin-3-yl)acetic acid **7-8***. Prepared by general procedure for the synthesis of **6** (Method A) and **7** (Method D). Yield 29.0%; Yellow powder; ^{1}H NMR (400 MHz, DMSO-d_6): δ 14.00–13.00 (br-s, 1H), 10.42 (s, 1H), 7.93 (s, 1H), 7.84 (s, 1H), 7.64 (d, 2H, J = 8.8 Hz), 7.54 (d, 1H, J = 8.4 Hz), 7.36 (t, 1H, J = 8.0 Hz), 7.18–7.14 (m, 3H), 4.84 (s, 2H), 4.22 (s, 2H); MS (ESI), m/z: 445.02 $[M-H]^-$.

*5.2.4.9. (Z)-Ethyl 2-(5-(4-(2-((3-chlorophenyl)amino)-2-oxoethoxy)benzylidene)-2,4-dioxothiazolidin-3-yl)acetate **7-9***. Prepared by general procedure for the synthesis of **6** (Method A) and **7** (Method D). Yield 49.5%; White powder; ^{1}H NMR (400 MHz, DMSO-d_6): δ ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.37 (s, 1H), 7.98 (s, 1H), 7.84 (s, 1H), 7.66 (d, 2H, J = 8.8 Hz), 7.53 (d, 1H, J = 8.0 Hz), 7.37 (t, 1H, J = 8.0 Hz), 7.19–7.15 (m, 3H), 4.85 (s, 2H), 4.49 (s, 2H), 4.17 (q, 2H, J = 7.2 Hz), 1.21 (t, 3H, J = 7.2 Hz); MS (ESI), m/z: 473.05 $[M-H]^-$.

5.2.4.10. (Z)-Methyl 3-(5-(4-(2-((3-chlorophenyl)amino)-2-oxoethoxy)benzylidene)-2,4-dioxothiazolidin-3-yl)propanoate ***7-10***. Prepared by general procedure for the synthesis of **6** (Method A) and **7** (Method D). Yield 69.3%; White powder; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.36 (s, 1H), 7.90 (s, 1H), 7.83 (s, 1H), 7.63 (d, 2H, J = 8.8 Hz), 7.52 (d, 1H, J = 8.0 Hz), 7.36 (t, 1H, J = 8.0 Hz), 7.18–7.15 (m, 3H), 4.84 (s, 2H), 3.88 (t, 2H, J = 7.2 Hz), 3.59 (s, 3H), 2.68 (t, 2H, J = 7.2 Hz); MS (ESI), m/z: 473.01 [M−H]$^-$.

5.2.4.11. (Z)-N-(3-Chlorophenyl)-2-(4-((3-(methoxymethyl)-2,4-dioxothiazolidin-5-ylidene)methyl)phenoxy)acetamide ***7-11***. Prepared by general procedure for the synthesis of **6** (Method A) and **7** (Method D). Yield 45.9%; Yellow powder; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.36 (s, 1H), 7.94 (s, 1H), 7.84 (s, 1H), 7.65 (d, 2H, J = 8.0 Hz), 7.30 (d, 1H, J = 8.4 Hz), 7.36 (t, 1H, J = 8.0 Hz), 7.19–7.14 (m, 3H), 4.98 (s, 2H), 4.84 (s, 2H), 3.30 (s, 3H); MS (ESI), m/z: 431.10 [M−H]$^-$.

5.2.4.12. (E)-Ethyl 4-((Z)-5-(4-(2-((3-chlorophenyl)amino)-2-oxoethoxy)benzylidene)-2,4-dioxothiazolidin-3-yl)but-2-enoate ***7-12***. Prepared by general procedure for the synthesis of **6** (Method A) and **7** (Method D). Yield 78.9%; White powder; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.37 (s, 1H), 7.92 (s, 1H), 7.83 (s, 1H), 7.64 (d, 2H, J = 8.8 Hz), 7.53 (d, 1H, J = 8.4 Hz), 7.37 (t, 1H, J = 8.0 Hz), 7.19–7.15 (m, 3H), 6.90–6.83 (tt, 1H, J = 4.8 Hz, J = 8.0 Hz), 5.95–5.91 (m, 1H), 4.84 (s, 2H), 4.44 (d, 2H, J = 3.2 Hz), 4.12 (q, 2H, J = 6.0 Hz), 1.12 (t, 3H, J = 6.0 Hz); MS (ESI), m/z: 517.01 [M−H]$^-$.

5.2.4.13. (Z)-N-(3-Chlorophenyl)-2-(4-((3-(2-(dimethylamino)ethyl)-2,4-dioxothiazolidin-5-ylidene)methyl)phenoxy)acetamide ***7-13***. Prepared by general procedure for the synthesis of **6** (Method A) and **7** (Method D). Yield 55.9%; Yellow powder; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.21 (s, 1H), 7.85 (s, 1H), 7.71 (t, 1H, J = 2.0 Hz), 7.52 (d, 2H, J = 8.8 Hz), 7.45 (dd, 1H, J = 0.8 Hz, J = 7.2 Hz), 7.29 (t, 1H, J = 8.0 Hz), 7.15 (dd, 1H, J = 0.8 Hz, J = 8.0 Hz), 7.09 (d, 2H, J = 8.8 Hz), 4.66 (s, 2H), 3.87 (t, 2H, J = 6.4 Hz), 2.58 (t, 2H, J = 6.4 Hz), 2.28 (s, 6H); MS (ESI), m/z: 476.05 [M−H]$^-$.

5.2.4.14. (Z)-N-(3-Chlorophenyl)-2-(4-((3-(3-(dimethylamino)-2-methylpropyl)-2,4-dioxothiazolidin-5-ylidene)methyl)phenoxy)acetamide ***7-14***. Prepared by general procedure for the synthesis of **6** (Method A) and **7** (Method D). Yield 76.8%; Yellow powder; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.29 (s, 1H), 7.81 (s, 1H), 7.70 (t, 1H, J = 2.0 Hz), 7.50 (d, 2H, J = 8.8 Hz), 7.48 (dd, 1H, J = 0.8 Hz, J = 7.2 Hz), 7.29 (t, 1H, J = 8.0 Hz), 7.15 (dd, 1H, J = 0.8 Hz, J = 8.0 Hz), 7.03 (d, 2H, J = 8.8 Hz), 4.67 (s, 2H), 3.96 (m, 1H), 3.87 (d, 2H, J = 6.4 Hz), 2.58 (d, 2H, J = 6.4 Hz), 2.28 (s, 6H), 2.11 (d, 3H, J = 6.0 Hz); MS (ESI), m/z: 486.10 [M−H]$^-$.

5.2.4.15. (Z)-N-(3-Chlorophenyl)-2-(4-((3-(2-(diethylamino)ethyl)-2,4-dioxothiazolidin-5-ylidene)methyl)phenoxy)acetamide ***7-15***. Prepared by general procedure for the synthesis of **6** (Method A) and **7** (Method D). Yield 47.8%; Yellow powder; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.30 (s, 1H), 7.85 (s, 1H), 7.71 (t, 1H, J = 2.0 Hz), 7.52 (d, 2H, J = 8.8 Hz), 7.45 (dd, 1H, J = 0.8 Hz, J = 7.2 Hz), 7.29 (t, 1H, J = 8.0 Hz), 7.15 (dd, 1H, J = 0.8 Hz, J = 8.0 Hz), 7.09 (d, 2H, J = 8.8 Hz), 4.66 (s, 2H), 3.87 (t, 2H, J = 6.4 Hz), 2.58 (t, 2H, J = 6.4 Hz), 2.28 (s, 6H); MS (ESI), m/z: 486.05 [M−H]$^-$.

5.2.4.16. (Z)-N-(3-Chlorophenyl)-2-(4-((2,4-dioxo-3-(2-(pyrrolidin-1-yl)ethyl)thiazolidin-5-ylidene)methyl)phenoxy)acetamide ***7-16***. Prepared by general procedure for the synthesis of **6** (Method A) and **7** (Method D). Yield 55.9%; Light yellow powder; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.38 (s, 1H), 9.04 (br-s, 1H), 7.94 (s, 1H), 7.84 (s, 1H), 7.65 (d, 2H, J = 8.4 Hz), 7.51 (d, 1H, J = 8.0 Hz), 7.37 (t, 1H, J = 8.0 Hz), 7.19–7.15 (m, 3H), 4.85 (s, 2H), 3.98 (s, 2H), 3.56–3.34 (m, 4H), 3.11 (s, 2H), 1.97–1.69 (m, 4H); MS (ESI), m/z: 484.10 [M−H]$^-$.

5.2.4.17. (Z)-N-(3-Chlorophenyl)-2-(4-((2,4-dioxo-3-(2-(piperidin-1-yl)ethyl)thiazolidin-5-ylidene)methyl)phenoxy)acetamide ***7-17***. Prepared by general procedure for the synthesis of **6** (Method A) and **7** (Method D). Yield 36.0%; Yellow powder; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.20 (s, 1H), 7.85 (s, 1H), 7.72 (s, 1H), 7.53 (d, 2H, J = 8.8 Hz), 7.45 (d, 1H, J = 8.0 Hz), 7.29 (t, 1H, J = 8.4 Hz), 7.15 (d, 1H, J = 8.0 Hz), 7.09 (d, 2H, J = 8.8 Hz), 4.67 (s, 2H), 3.88 (t, 2H, J = 6.8 Hz), 2.58 (t, 2H, J = 6.8 Hz), 2.45 (m, 4H), 1.54–1.52 (m, 4H), 1.42–1.40 (m, 2H); MS (ESI), m/z: 498.05 [M−H]$^-$.

5.2.4.18. (Z)-N-(3-Chlorophenyl)-2-(4-((3-(2-morpholinoethyl)-2,4-dioxothiazolidin-5-ylidene)methyl)phenoxy)acetamide ***7-18***. Prepared by general procedure for the synthesis of **6** (Method A) and **7** (Method D). Yield 81.1%; Light yellow powder; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.20 (s, 1H), 7.85 (s, 1H), 7.71 (s, 1H), 7.54 (d, 2H, J = 8.4 Hz), 7.45 (d, 1H, J = 7.6 Hz), 7.31–7.26 (m, 2H), 7.15 (d, 1H, J = 8.0 Hz), 7.10 (d, 2H, J = 8.8 Hz), 4.67 (s, 2H), 3.88 (t, 2H, J = 6.4 Hz), 3.65 (t, 4H, J = 4.0 Hz), 2.62 (t, 2H, J = 6.4 Hz), 2.50 (m, 4H); MS (ESI), m/z: 500.01 [M−H]$^-$.

5.2.4.19. (Z)-2-(4-((3-Benzyl-2,4-dioxothiazolidin-5-ylidene)methyl)phenoxy)-N-(3-chlorophenyl)acetamide ***7-19***. Prepared by general procedure for the synthesis of **6** (Method B) and **7** (Method E). Yield 56.9%; White powder; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.37 (s, 1H), 7.94 (s, 1H), 7.84 (s, 1H), 7.64 (d, 2H, J = 8.8 Hz), 7.53 (d, 1H, J = 8.4 Hz), 7.39–7.30 (m, 6H), 7.18–7.15 (m, 3H), 4.84 (s, 4H). MS (ESI), m/z: 477.07 [M−H]$^-$.

5.2.4.20. (Z)-N-(3-Chlorophenyl)-2-(4-((3-(2-methylbenzyl)-2,4-dioxothiazolidin-5-ylidene)methyl)phenoxy)acetamide ***7-20***. Prepared by general procedure for the synthesis of **6** (Method B) and **7** (Method E). Yield 44.3%; White powder; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.37 (s, 1H), 7.95 (s, 1H), 7.84 (s, 1H), 7.65 (d, 2H, J = 8.8 Hz), 7.53 (d, 1H, J = 8.0 Hz), 7.36 (t, 1H, J = 8.0 Hz), 7.20–7.15 (m, 6H), 7.03 (d, 1H, J = 6.8 Hz), 4.84 (s, 2H), 4.82 (s, 2H), 2.63 (s, 3H); MS (ESI), m/z: 491.10 [M−H]$^-$.

5.2.4.21. (Z)-N-(3-Chlorophenyl)-2-(4-((3-(2-fluorobenzyl)-2,4-dioxothiazolidin-5-ylidene)methyl)phenoxy)acetamide ***7-21***. Prepared by general procedure for the synthesis of **6** (Method B) and **7** (Method E). Yield 21.0%; Light yellow powder; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.37 (s, 1H), 7.94 (s, 1H), 7.84 (t, 1H, J = 2.0 Hz), 7.63 (d, 2H, J = 9.2 Hz), 7.54–7.51 (m, 1H), 7.39–7.35 (m, 3H), 7.21–7.14 (m, 5H), 4.84 (s, 2H), 4.20 (s, 2H); MS (ESI), m/z: 495.05 [M−H]$^-$.

5.2.4.22. (Z)-N-(3-Chlorophenyl)-2-(4-((3-(3-fluorobenzyl)-2,4-dioxothiazolidin-5-ylidene)methyl)phenoxy)acetamide ***7-22***. Prepared by general procedure for the synthesis of **6** (Method B) and **7** (Method E). Yield 33.9%; White powder; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.37 (s, 1H), 7.95 (s, 1H), 7.84 (t, 1H, J = 2.0 Hz), 7.64 (d, 2H, J = 8.8 Hz), 7.53 (d, 1H, J = 8.0 Hz), 7.43–7.35 (m, 2H), 7.18–7.13 (m, 5H), 4.89 (s, 2H), 4.84 (s, 2H); MS (ESI), m/z: 495.03 [M−H]$^-$.

5.2.4.23. (Z)-N-(3-Chlorophenyl)-2-(4-((3-(4-fluorobenzyl)-2,4-dioxothiazolidin-5-ylidene)methyl)phenoxy)acetamide ***7-23***. Prepared by general procedure for the synthesis of **6** (Method B) and **7** (Method E). Yield 43.5%; White powder; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.37 (s, 1H), 7.94 (s, 1H), 7.84 (s, 1H), 7.64 (d, 2H, J = 8.8 Hz), 7.53 (d, 1H, J = 8.8 Hz), 7.39–7.31 (m, 2H), 7.25–7.15 (m,

5H), 4.89 (s, 2H), 4.84 (s, 2H); MS (ESI), *m/z*: 495.06 [M−H]$^-$.

5.2.4.24. (Z)-2-(4-((3-(2-Chlorobenzyl)-2,4-dioxothiazolidin-5-ylidene)methyl)phenoxy)-N-(3-chlorophenyl)acetamide ***7-24***.
Prepared by general procedure for the synthesis of **6** (Method B) and **7** (Method E). Yield 45.9%; White powder; ^{1}H NMR (400 MHz, DMSO-d$_6$): δ 10.37 (s, 1H), 7.96 (s, 1H), 7.84 (s, 1H), 7.65 (d, 2H, *J* = 8.4 Hz), 7.51 (t, 1H, *J* = 8.4 Hz), 7.39–7.31 (m, 3H), 7.24–7.14 (m, 4H), 4.90 (s, 2H), 4.85 (s, 2H); MS (ESI), *m/z*: 511.03 [M−H]$^-$.

5.2.4.25. (Z)-2-(4-((3-(3-Chlorobenzyl)-2,4-dioxothiazolidin-5-ylidene)methyl)phenoxy)-N-(3-chlorophenyl)acetamide ***7-25***.
Prepared by general procedure for the synthesis of **6** (Method B) and **7** (Method E). Yield 57.5%; White powder; ^{1}H NMR (400 MHz, DMSO-d$_6$): δ 10.36 (s, 1H), 7.94 (s, 1H), 7.84 (s, 1H), 7.63 (d, 2H, *J* = 8.4 Hz), 7.52 (d, 1H, *J* = 8.0 Hz), 7.39–7.34 (m, 4H), 7.27 (m, 1H), 7.18–7.14 (m, 3H), 4.84 (s, 4H); MS (ESI), *m/z*: 511.00 [M−H]$^-$.

5.2.4.26. (Z)-2-(4-((3-(4-Chlorobenzyl)-2,4-dioxothiazolidin-5-ylidene)methyl)phenoxy)-N-(3-chlorophenyl)acetamide ***7-26***.
Prepared by general procedure for the synthesis of **6** (Method B) and **7** (Method E). Yield 69.0%; White powder; ^{1}H NMR (400 MHz, DMSO-d$_6$): δ 10.36 (s, 1H), 7.93 (s, 1H), 7.83 (s, 1H), 7.63 (d, 2H, *J* = 8.4 Hz), 7.52 (d, 1H, *J* = 8.4 Hz), 7.43–7.33 (m, 5H), 7.18–7.14 (m, 3H), 4.84 (s, 2H), 4.82 (s, 2H); MS (ESI), *m/z*: 511.03 [M−H]$^-$.

5.2.4.27. (Z)-2-(4-((3-(4-Bromobenzyl)-2,4-dioxothiazolidin-5-ylidene)methyl)phenoxy)-N-(3-chlorophenyl)acetamide ***7-27***.
Prepared by general procedure for the synthesis of **6** (Method B) and **7** (Method E). Yield 56.0%; White powder; ^{1}H NMR (400 MHz, DMSO-d$_6$): δ 10.36 (s, 1H), 7.93 (s, 1H), 7.83 (s, 1H), 7.63 (d, 2H, *J* = 8.4 Hz), 7.56–7.51 (m, 3H), 7.36 (t, 1H, *J* = 8.0 Hz), 7.27 (d, 2H, *J* = 8.0 Hz), 7.18–7.14 (m, 3H), 4.84 (s, 2H), 4.80 (s, 2H); MS (ESI), *m/z*: 554.99 [M−H]$^-$.

5.2.4.28. (Z)-N-(3-Chlorophenyl)-2-(4-((3-(4-cyanobenzyl)-2,4-dioxothiazolidin-5-ylidene)methyl)phenoxy)acetamide ***7-28***.
Prepared by general procedure for the synthesis of **6** (Method B) and **7** (Method E). Yield 80.0%; Yellow solid; ^{1}H NMR (400 MHz, DMSO-d$_6$): δ 10.37 (s, 1H), 7.94 (s, 1H), 7.83 (m, 3H), 7.64 (d, 2H, *J* = 8.8 Hz), 7.53–7.49 (m, 3H), 7.36 (t, 1H, *J* = 8.0 Hz), 7.18–7.14 (m, 3H), 4.92 (s, 2H), 4.84 (s, 2H); MS (ESI), *m/z*: 502.05 [M−H]$^-$.

5.2.4.29. (Z)-N-(3-Chlorophenyl)-2-(4-((3-(4-nitrobenzyl)-2,4-dioxothiazolidin-5-ylidene)methyl)phenoxy)acetamide ***7-29***.
Prepared by general procedure for the synthesis of **6** (Method B) and **7** (Method E). Yield 50.2%; White powder; ^{1}H NMR (400 MHz, DMSO-d$_6$): δ 10.37 (s, 1H), 8.22 (d, 2H, *J* = 9.2 Hz), 7.96 (s, 1H), 7.84 (s, 1H), 7.65 (d, 2H, *J* = 8.8 Hz), 7.59 (d, 2H, *J* = 8.8 Hz), 7.52 (d, 1H, *J* = 9.6 Hz), 7.37 (t, 1H, *J* = 8.0 Hz), 7.19–7.15 (m, 3H), 4.98 (s, 2H), 4.85 (s, 2H); MS (ESI), *m/z*: 522.04 [M−H]$^-$.

5.2.4.30. (Z)-methyl 4-((5-(4-(2-((3-Chlorophenyl)amino)-2-oxoethoxy)benzylidene)-2,4-dioxothiazolidin-3-yl)methyl)benzoate ***7-30***. Prepared by general procedure for the synthesis of **6** (Method B) and **7** (Method E). Yield 32.9%; White powder; ^{1}H NMR (400 MHz, DMSO-d$_6$): δ 10.37 (s, 1H), 7.95–7.93 (m, 3H), 7.84 (s, 1H), 7.64 (d, 2H, *J* = 8.4 Hz), 7.53 (d, 1H, *J* = 9.2 Hz), 7.45 (d, 1H, *J* = 8.4 Hz), 7.37 (t, 1H, *J* = 8.0 Hz), 7.19–7.14 (m, 3H), 4.91 (s, 2H), 4.84 (s, 2H), 3.84 (s, 3H); MS (ESI), *m/z*: 535.07 [M−H]$^-$.

5.2.4.31. (Z)-4-((5-(4-(2-((3-Chlorophenyl)amino)-2-oxoethoxy)benzylidene)-2,4-dioxothiazolidin-3-yl)methyl)benzoic acid ***7-31***.
Prepared by general procedure for the synthesis of **6** (Method C) and **7** (Method F). Yield 75.0%; White powder; ^{1}H NMR (400 MHz, DMSO-d$_6$): δ 12.98 (s, 1H), 10.36 (s, 1H), 7.95–7.91 (m, 3H), 7.83 (t, 1H, *J* = 1.6 Hz), 7.64 (d, 2H, *J* = 8.8 Hz), 7.30 (d, 1H, *J* = 8.0 Hz), 7.42 (d, 2H, *J* = 8.8 Hz), 7.36 (t, 1H, *J* = 8.0 Hz), 7.19–7.14 (m, 3H), 4.90 (s, 3H), 4.84 (s, 3H); MS (ESI), *m/z*: 522.02 [M−H]$^-$.

5.2.4.32. (Z)-N-(3-Chlorophenyl)-2-(4-((2,4-dioxo-3-(2-oxo-2-(phenylamino)ethyl)thiazolidin-5-ylidene)methyl)phenoxy)acetamide ***7-32***. Prepared by general procedure for the synthesis of **6** (Method C) and **7** (Method F). Yield 34.0%; White powder; ^{1}H NMR (400 MHz, DMSO-d$_6$): δ 10.23 (s, 1H), 9.96 (s, 1H), 7.85 (s, 1H), 7.75 (s, 1H), 7.65–7.56 (m, 2H), 7.34–7.26 (m, 3H), 6.90 (s, 1H), 7.39–7.30 (m, 5H), 4.66 (s, 2H), 4.43 (s, 2H); MS (ESI), *m/z*: 520.05 [M−H]$^-$.

5.2.4.33. (Z)-N-(3-Chlorophenyl)-2-(4-((2,4-dioxo-3-(2-oxo-2-(p-tolylamino)ethyl)thiazolidin-5-ylidene)methyl)phenoxy)acetamide ***7-33***. Prepared by general procedure for the synthesis of **6** (Method C) and **7** (Method F). Yield 10.9%; White powder; ^{1}H NMR (400 MHz, DMSO-d$_6$): δ 10.37 (s, 1H), 10.31 (s, 1H), 7.76 (s, 1H), 7.84 (t, 1H, *J* = 2.0 Hz), 7.66 (d, 2H, *J* = 8.8 Hz), 7.53 (d, 1H, *J* = 9.2 Hz), 7.43 (d, 2H, *J* = 8.4 Hz), 7.37 (t, 1H, *J* = 8.0 Hz), 7.29 (d, 1H, *J* = 8.0 Hz), 7.19 (d, 2H, *J* = 9.2 Hz), 7.13 (d, 2H, *J* = 8.4 Hz), 4.85 (s, 2H), 4.49 (s, 2H), 2.25 (s, 3H); MS (ESI), *m/z*: 552.05 [M−H]$^-$.

5.2.4.34. (Z)-N-(4-Acetylphenyl)-2-(5-(4-(2-((3-chlorophenyl)amino)-2-oxoethoxy)benzylidene)-2,4-dioxothiazolidin-3-yl)acetamide ***7-34***. Prepared by general procedure for the synthesis of **6** (Method C) and **7** (Method F). Yield 43.1%; Brown solid; ^{1}H NMR (400 MHz, DMSO -d$_6$): δ 10.78 (s, 1H), 10.38 (s, 1H), 7.97 (s, 2H), 7.84 (s, 1H), 7.45 (s, 1H), 7.71–7.66 (m, 4H), 7.53 (d, 1H, *J* = 8.4 Hz), 7.37 (t, 1H, *J* = 8.0 Hz), 7.20–7.15 (m, 3H), 4.85 (s, 2H), 4.56 (s, 2H), 2.54 (s, 3H); MS (ESI), *m/z*: 562.06 [M−H]$^-$.

5.2.4.35. (Z)-N-(3-Chlorophenyl)-2-(4-((3-(2-(naphthalen-1-ylamino)-2-oxoethyl)-2,4-dioxothiazolidin-5-ylidene)methyl)phenoxy)acetamide ***7-35***. Prepared by general procedure for the synthesis of **6** (Method C) and **7** (Method F). Yield 45.0%; Brown solid; ^{1}H NMR (400 MHz, DMSO-d$_6$): δ 10.41 (s, 1H), 10.37 (s, 1H), 8.11 (d, 1H, *J* = 8.0 Hz), 7.98–7.95 (m, 2H), 7.83 (s, 1H), 7.80 (d, 1H, *J* = 8.0 Hz), 7.68–7.64 (m, 3H), 7.61–7.56 (m, 2H), 7.56–7.48 (m, 2H), 7.36 (t, 1H, *J* = 8.0 Hz), 7.19–7.14 (m, 3H), 4.84 (s, 2H), 4. 69 (s, 2H); MS (ESI), *m/z*: 570.10 [M−H]$^-$.

5.2.4.36. (Z)-N-Benzyl-2-(5-(4-(2-((3-chlorophenyl)amino)-2-oxoethoxy)benzylidene)-2,4-dioxothiazolidin-3-yl)acetamide ***7-36***. Prepared by general procedure for the synthesis of **6** (Method C) and **7** (Method F). Yield 55.0%; White powder; ^{1}H NMR (400 MHz, DMSO-d$_6$): δ 10.37 (s, 1H), 8.01 (t, 1H, *J* = 6.0 Hz), 7.94 (s, 1H), 7.84 (s, 1H), 7.65 (d, 2H, *J* = 8.8 Hz), 7.53 (d, 1H, *J* = 8.0 Hz), 7.37–7.34 (m, 3H), 7.26 (m, 3H), 7.19–7.15 (m, 3H), 4.84 (s, 2H), 4.34 (s, 2H), 4.31 (d, 2H, *J* = 6.0 Hz); MS (ESI), *m/z*: 552.04 [M−H]$^-$.

5.2.4.37. (Z)-2-(5-(4-(2-((3-Chlorophenyl)amino)-2-oxoethoxy)benzylidene)-2,4-dioxothiazolidin-3-yl)-N-methyl-N-phenylacetamide ***7-37***. Prepared by general procedure for the synthesis of **6** (Method C) and **7** (Method F). Yield 34.0%; White powder; ^{1}H NMR (400 MHz, DMSO-d$_6$): δ 10.37 (s, 1H), 7.90 (s, 1H), 7.84 (t, 1H, *J* = 2.0 Hz), 7.64 (d, 2H, *J* = 9.2 Hz), 7.55–7.47 (m, 5H), 7.37 (t, 2H, *J* = 8.0 Hz), 7.18–7.14 (m, 3H), 4.84 (s, 2H), 4. 12 (s, 2H), 3.20 (s, 3H); MS (ESI), *m/z*: 552.07 [M−H]$^-$.

5.2.4.38. (Z)-N-(3-Chlorophenyl)-2-(4-((2,4-dioxo-3-(pyridin-2-ylmethyl)thiazolidin-5-ylidene)methyl)phenoxy)acetamide ***7-38***. Prepared by general procedure for the synthesis of **6** (Method C) and **7** (Method F). Yield 44.5%; White powder; ^{1}H NMR (400 MHz, DMSO-d$_6$): δ 10.37 (s, 1H), 8.56 (s, 1H), 8.52 (d, 1H, *J* = 4.4 Hz), 7.95

(s, 1H), 7.84 (s, 1H), 7.73 (d, 1H, J = 8.0 Hz), 7.64 (d, 2H, J = 8.4 Hz), 7.53 (d, 1H, J = 8.0 Hz), 7.38 (q, 2H, J = 8.0 Hz), 7.19–7.15 (m, 3H), 4.88 (s, 2H), 4.84 (s, 2H); MS (ESI), m/z: 478.00 $[M-H]^-$.

5.2.4.39. (Z)-N-(3-Chlorophenyl)-2-(4-((2,4-dioxo-3-(pyridin-3-ylmethyl)thiazolidin-5-ylidene)methyl)phenoxy)acetamide ***7-39***. Prepared by general procedure for synthesis of **6** (Method C) and **7** (Method F). Yield 31.0%; Light yellow powder; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.55 (d, 1H, J = 4.8 Hz), 8.20 (s, 1H), 7.90 (s, 1H), 7.72 (s, 1H), 7.67 (t, 1H, J = 6.8 Hz), 7.54 (d, 2H, J = 8.4 Hz), 7.45 (d, 1H, J = 8.0 Hz), 7.31–7.28 (m, 2H), 7.22–7.14 (m, 2H), 7.10 (d, 2H, J = 8.4 Hz), 5.07 (s, 2H), 4.67 (s, 2H); MS (ESI), m/z: 478.10 $[M-H]^-$.

5.2.4.40. (Z)-N-(3-Chlorophenyl)-2-(3-((2,4-dioxothiazolidin-5-ylidene)methyl)phenoxy)acetamide ***7-40***. Prepared by general procedure for the synthesis of **7** (Method E). Yield 59.0%; White powder; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.54 (s, 1H), 11.97 (s, 1H), 10.35 (s, 1H), 7.84 (s, 1H), 7.76 (s, 1H), 7.58 (d, J = 8.8 Hz, 2H), 7.52 (m 3H), 7.17 (m, 1H), 7.15 (m, 1H), 4.83 (s, 2H); MS (ESI), m/z: 387.04 $[M-H]^-$.

5.2.4.41. (Z)-N-(3-Chlorophenyl)-2-(5-((2,4-dioxothiazolidin-5-ylidene)methyl)-2-methoxyphenoxy)acetamide ***7-41***. Prepared by general procedure for the synthesis of **7** (Method E). Yield 84.1%; Yellow powder; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.50 (s, 1H), 10.40 (s, 1H), 7.84 (s, 1H), 7.71 (s, 1H), 7.50 (d, 1H, J = 8.0 Hz), 7.36 (t, 1H, J = 8.0 Hz), 7.27 (d, 1H, J = 8.4 Hz), 7.18 (d, 1H, J = 8.4 Hz), 7.14 (br-s, 2H), 4.79 (s, 2H), 3.88 (s, 3H); MS (ESI), m/z: 417.03 $[M-H]^-$.

5.2.4.42. (Z)-N-(3-Chlorophenyl)-2-(4-((2,4-dioxothiazolidin-5-ylidene)methyl)-2-methoxyphenoxy)acetamide ***7-42***. Prepared by general procedure for the synthesis of **7** (Method E). Yield 69.0%; Light yellow powder; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.55 (s, 1H), 10.38 (s, 1H), 7.82 (s, 1H), 7.76 (s, 1H), 7.48 (d, 1H, J = 7.9 Hz), 7.36 (t, 1H, J = 8.0 Hz), 7.26 (s, 1H), 7.17–7.14 (m, 2H), 7.07 (d, 1H, J = 8.4 Hz), 4.82 (s, 2H), 3.83 (s, 3H); MS (ESI), m/z: 417.02 $[M-H]^-$.

5.2.4.43. (Z)-N-(3-Chlorophenyl)-2-(4-((2,4-dioxothiazolidin-5-ylidene)methyl)-2-ethoxyphenoxy)acetamide ***7-43***. Prepared by general procedure for the synthesis of **7** (Method E). Yield 39.0%; Yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.55 (s, 1H), 10.37 (s, 1H), 7.50 (s, 1H), 7.48 (s, 1H), 7.36 (t, 1H, J = 8.0 Hz), 7.24 (d, 1H, J = 1.2 Hz), 7.17–7.14 (m, 2H), 7.06 (d, 1H, J = 8.4 Hz), 4.83 (s, 2H), 4.11 (q, 2H, J = 7.2 Hz), 1.38 (t, 3H, J = 7.2 Hz); MS (ESI), m/z: 431.05 $[M-H]^-$.

5.2.4.44. (Z)-N-(3-Chlorophenyl)-2-(4-((2,4-dioxothiazolidin-5-ylidene)methyl)-2,6-dimethoxyphenoxy)acetamide ***7-44***. Prepared by general procedure for the synthesis of **7** (Method E). Yield 20.0%; Yellow powder; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.64 (s, 1H), 10.05 (s, 1H), 7.90 (t, 1H, J = 2.0 Hz), 7.77 (s, 1H), 7.55 (d, 1H, J = 8.0 Hz), 7.38 (t, 1H, J = 8.0 Hz), 7.17 (dd, 1H, J = 0.8 Hz, J = 8.0 Hz), 6.98 (s, 2H), 4.59 (s, 2H), 3.87 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.2, 167.7, 166.7, 159.6, 140.0, 132.2, 131.9, 130.7, 126.4, 123.7, 121.0, 119.4, 118.2, 115.8, 67.2, 57.2; MS (ESI), m/z: 447.03 $[M-H]^-$.

5.2.4.45. (Z)-N-(3-Chlorophenyl)-2-(4-((2,4-dioxothiazolidin-5-ylidene)methyl)-2-fluorophenoxy)acetamide ***7-45***. Prepared by general procedure for the synthesis of **7** (Method E). Yield 40.0%; Light yellow powder; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.62 (s, 1H), 10.45 (s, 1H), 7.81 (s, 1H), 7.74 (s, 1H), 7.55 (dd, 1H, J = 1.6 Hz, J = 11.2 Hz), 7.47 (d, 1H, J = 7.6 Hz), 7.40–7.33 (m, 2H), 7.27 (t, 1H, J = 8.8 Hz), 7.15 (d, 1H, J = 8.0 Hz), 4.94 (s, 2H); MS (ESI), m/z: 405.01 $[M-H]^-$.

5.2.4.46. (Z)-N-(3-Chlorophenyl)-2-(4-(1-(2,4-dioxothiazolidin-5-ylidene)ethyl)phenoxy)acetamide ***7-46***. Prepared by general procedure for the synthesis of **7** (Method F). Yield 50.5%; Brown solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.36 (s, 1H), 9.96 (s, 1H), 7.76 (s, 1H), 7.75 (s, 1H), 7.41 (d, 2H, J = 8.4 Hz), 7.36 (d, 1H, J = 8.0 Hz), 7.33 (t, 1H, J = 8.2 Hz), 7.18 (d, 2H, J = 8.4 Hz), 4.66 (s, 2H), 2.43 (s, 3H); MS (ESI), m/z: 401.03 $[M-H]^-$.

5.2.4.47. (Z)-N-(3-Chlorophenyl)-3-(4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenoxy)propanamide ***7-47***. Prepared by general procedure for the synthesis of **7** (Method D). Yield 31.9%; White powder; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.27 (s, 1H), 10.00 (s, 1H), 7.94 (s, 1H), 7.72 (s, 1H), 7.62–7.31 (m, 3H), 7.15 (s, 1H), 7.00 (d, 1H, J = 8.0 Hz), 6.97 (d, 2H, J = 8.0 Hz), 4.32 (t, 2H, J = 6.0 Hz), 2.64 (t, 2H, J = 6.0 Hz); MS (ESI), m/z: 401.01 $[M-H]^-$.

5.2.4.48. (Z)-N-(3-Chlorophenyl)-4-(4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenoxy)butanamide ***7-48***. Prepared by general procedure for the synthesis of **7** (Method D). Yield 59.0%; White powder; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.31 (s, 1H), 10.03 (s, 1H), 7.97 (s, 1H), 7.75 (s, 1H), 7.65 (d, 1H, J = 1.6 Hz), 7.34 (s, 1H), 7.02 (d, 1H, J = 1.6 Hz), 4.11 (t, J = 5.6 Hz, 2H), 2.80 (t, 2H, J = 5.6 Hz), 2.23–2.15 (m, 2H); MS (ESI), m/z: 415.00 $[M-H]^-$.

5.2.5. Synthesis of compound ***7-49***

Step 1: 3-Chloroaniline (6.38 g, 50 mmol), N-Boc-Gly (9.28 g, 53 mmol), EDCI (11.50 g, 60 mmol) was dissolved into DCM (100 mL) at 0 °C and allowed to warm up to room temperature overnight. Then, the solvent was evaporated and extracted by EtOAc (3 × 50 mL) and water (3 × 50 mL). The organic layers was combined and washed by 0.5 N HCl (2 × 15 mL), $NaHCO_3$ (2 × 15 mL), water (2 × 15 mL), brine (2 × 10 mL), and dried by anhydrous Na_2SO_4. The solvent was removed to obtain the crude product **2-49** without purification. Yield 95.0%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.60 (s, 1H), 7.63 (s, 1H), 7.36 (d, 1H, J = 8.0 Hz), 7.22 (t, 1H, J = 8.0 Hz), 7.07 (d, 1H, J = 8.0 Hz), 5.47 (s, 1H), 3.95 (d, 2H, J = 6.4 Hz), 1.48 (s, 9H); MS (ESI), m/z: 283.00 $[M-H]^-$.

Step 2: In ice bath, **2-49** (14.2 g, 50 mmol) was dissolved into anhydrous THF (60 mL) and $LiAlH_4$ (4.9 g, 125 mmol) was slowly added into the mixture at 0 °C and allowed to warm up to room temperature overnight. Then, 250 mL water added and the solvent was filtered. The layer was washed by EtOAc (3 × 50 mL), water (3 × 50 mL) and brine (2 × 30 mL), dried by anhydrous Na_2SO_4. The solvent was removed to obtain the white solid **3-49**. Yield 91.2%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.06 (t, 1H, J = 8.0 Hz), 6.65 (d, 1H, J = 8.0 Hz), 6.56 (s, 1H), 6.46 (dd, 1H, J = 8.0 Hz, J = 3.0 Hz), 4.83 (s, 1H), 4.21 (s, 1H), 3.37 (t, 2H, J = 5.8 Hz), 3.24 (t, 2H, J = 6.0 Hz), 1.45 (s, 9H); MS (ESI), m/z: 269.02 $[M-H]^-$.

Step 3: In ice bath, **3-49** (5.6 g, 20.6 mmol) and $NaHCO_3$ (2.6 g, 30.9 mmol) was dissolved into DCM (50 mL) and stirred for 10 min. Then, 2-chloroacetyl chloride (3.49 g, 30.9 mmol) was added slowly and allowed to warm up to room temperature overnight. Then, 100 mL water added and the solvent was filtered. The layer was washed by EtOAc (3 × 20 mL), water (3 × 20 mL) and brine (2 × 20 mL), dried by anhydrous Na_2SO_4. The solvent was removed to obtain the white solid **4-49**. Yield 79.6%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.42–7.13 (m, 2H), 7.30–7.27 (m, 1H), 7.21 (m, 1H), 4.91 (s, 2H), 3.85 (s, 2H), 3.87–3.83 (m, 2H), 3.35 (d, 2H, J = 6.4 Hz), 1.42 (s, 9H); MS (ESI), m/z: 345.01 $[M-H]^-$.

Step 4: **4-49** (1.5 g, 4.26 mmol), 4-hydroxybenzaldehyde (0.52 g, 4.26 mmol), K_2CO_3 (0.88 g, 6.39 mmol) and KI (1.06 g, 6.39 mmol) were dissolved in anhydrous acetone (100 mL). The reaction mixture was refluxed overnight and then cooled to room temperature. Then the K_2CO_3 solid was filtered and the acetone solution was removed under reduced pressure to obtain the crude products.

The residue was purified by silica gel column chromatography to give the white solid **5-49**. Yield 82.0%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.88 (s, 1H), 7.80 (d, 2H, J = 8.0 Hz), 7.44 (d, 2H, J = 8.0 Hz), 7.41 (s, 1H), 7.21 (s, 1H), 6.87 (d, 2H, J = 8.0 Hz), 4.90 (s, 1H), 4.50 (s, 2H), 3.86 (t, 2H, J = 5.2 Hz), 3.35 (d, 2H, J = 5.2 Hz), 1.43 (s, 9H); MS (ESI), m/z: 431.01 [M−H]$^-$.

Step 5: **5-49** (230 mg, 0.5 mmol), thiazolidine-2,4-dione (115 mg, 0.8 mmol), piperidine (83 mg, 0.8 mmol), benzoic acid (98 mg, 0.8 mmol), and 10 mL toluene/EtOH (1:1) were heated to reflux overnight. The mixture was filtered and washed by EtOH (2 × 10 mL), water (2 × 10 mL), and dried in vacuum at 40 °C for 24 h to afford light yellow solid **6-49**. Yield 48.9%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.86 (s, 1H), 7.69 (s, 1H), 7.51–7.32 (m, 5H), 7.23 (m, 1H), 6.85 (d, 2H, J = 8.0 Hz), 4.99 (s, 1H), 4.48 (s, 2H), 3.86 (t, 2H, J = 6.4 Hz), 3.36 (t, 2H, J = 6.4 Hz), 1.44 (s, 9H); MS (ESI), m/z: 530.10 [M−H]$^-$.

Step 6: In ice bath, **6-49** (53 mg, 0.1 mmol) was added in 5 mL TFA/DCM (1:1) and stirred for 2 h. The mixture was evaporated to dryness and dissolved into EtOAc and washed by $NaHCO_3$ (2 × 15 mL), water (2 × 15 mL), brine (2 × 15 mL) and dried by anhydrous Na_2SO_4. The solvent was evaporated to give the white solid **7-49**.

5.2.5.1. (Z)-N-(2-Aminoethyl)-N-(3-chlorophenyl)-2-(4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenoxy)acetamide ***7-49***. Yield 40.0%; White powder; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.52 (s, 1H), 7.74 (s, 1H), 7.70 (s, 1H), 7.51 (m, 5H), 6.96–6.95 (m, 2H) 4.51 (s, 2H), 3.66 (d, J = 6.0 Hz, 2H), 3.08 (d, J = 6.0 Hz, 2H); MS (ESI), m/z: 430.00 [M−H]$^-$.

5.2.6. Synthesis of compounds 8

Synthesis of **8a** [15]: 2-chloro-N-(pyridin-2-yl)acetamide (1 mmol), KSCN (2 mmol), and 10 mL acetone were refluxed for 5 h. The mixture was filtered and washed by water. The crude product was recystallized by EtOH to afford yellow solid **8a** (Yield 76%).

Synthesis of **8b** [17]: 1H-pyrrole-2,5-dione (1.0 mmol), PPh_3 (1.0 mmol) and 5 mL CH_3OH was heated to reflux for 1.5 h. The mixture was filtered and washed by CH_3OH (2 × 10 mL) to afford the **8b** (Yield 89.9%).

Synthesis of **8d-e** [16]: In ice bath, imidazolidine-2,4-dione (1.0 mmol) was dissolved in 10 mL DMF and NaH (1.5 mmol, 60%) was slowly added into the mixture. Then, (bromomethyl)benzene (1.1 mmol) was added and stirred overnight. The mixture was evaporated to dryness. The residue was purified by silica gel column chromatography to give **8d** and **8e**.

5.2.7. Synthesis of bioisosteric derivatives of thiazolidine-2,4-dione

5.2.7.1. (Z)-N-(3-Chlorophenyl)-2-(4-((2-imino-4-oxo-3-(pyridin-2-yl)thiazolidin-5-ylidene)methyl)phenoxy)acetamide ***7-50***. Procedure: **4a** (0.3 mmol), **8a** (0.36 mmol), anhydrous NaOAc (0.6 mmol) and 5 mL acetic acid were heated to 100 °C for 5 h. The mixture was filtered and washed by EtOH (2 × 10 mL), water (2 × 10 mL), and dried in vacuum at 40 °C for 24 h to afford **7-50**. Yield 21.0%; Brown solid; ^{1}H NMR (400 MHz, DMSO -d_6): δ 12.37 (br-s, 1H), 10.37 (s, 1H), 8.53 (s, 1H), 7.88–7.84 (m, 2H), 7.66 (d, 2H, J = 8.4 Hz), 7.62 (s, 1H), 7.54 (d, 1H, J = 8.4 Hz), 7.37 (t, 1H, J = 8.0 Hz), 7.22–7.12 (m, 5H), 4.83 (s, 2H), 4.11 (q, 2H, J = 7.2 Hz), 1.38 (t, 3H, J = 7.2 Hz); MS (ESI), m/z: 463.05 [M−H]$^-$.

5.2.7.2. (Z)-N-(3-Chlorophenyl)-2-(4-((2,5-dioxoimidazolidin-4-ylidene)methyl)phenoxy)acetamide ***7-51***. Procedure: **4a** (1.0 mmol), **8c** (1.0 mmol), pyrrolidine (cat.), and 10 mL EtOH were heated to reflux for 5 h. The mixture was filtered and washed by EtOH (2 × 10 mL), water (2 × 10 mL), and dried in vacuum at 40 °C for 24 h to afford **7-51**. Yield 43.0%; Yellow powder; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.19 (s, 1H), 10.46 (s, 1H), 10.31 (s, 1H), 7.85 (t, 1H, J = 2.0 Hz), 7.61 (d, 2H, J = 8.8 Hz), 7.54 (dd, 1H, J = 1.8 Hz, J = 8.4 Hz), 7.36 (t, 1H, J = 8.0 Hz), 7.15 (dd, 1H, J = 2.0 Hz, J = 8.0 Hz), 7.03 (d, 2H, J = 8.8 Hz), 4.78 (s, 2H); MS (ESI), m/z: 370.05 [M−H]$^-$.

5.2.7.3. (Z)-2-(4-((1-Benzyl-2,5-dioxoimidazolidin-4-ylidene)methyl)phenoxy)-N-(3-chlorophenyl)acetamide ***7-52***. Prepared by procedure for the synthesis of **7-51** using **8d**. Yield 19.5%; White powder; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.80 (s, 1H), 10.32 (s, 1H), 7.84 (s, 1H), 7.64 (d, 2H, J = 8.8 Hz), 7.54 (d, 1H, J = 8.4 Hz), 7.38–7.28 (m, 6H), 7.15 (d, 1H, J = 7.6 Hz), 7.04 (d, 2H, J = 8.4 Hz), 4.79 (s, 2H), 4.66 (s, 2H); MS (ESI), m/z: 460.10 [M−H]$^-$.

5.2.7.4. (Z)-N-(3-Chlorophenyl)-2-(4-((1,3-dibenzyl-2,5-dioxoimidazolidin-4-ylidene)methyl)phenoxy)acetamide ***7-53***. Prepared by procedure for the synthesis of **7-51** using **8e**. Yield 40.0%; White powder; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.30 (s, 1H), 7.94 (s, 1H), 7.92 (s, 1H), 7.83 (s, 1H), 7.53 (d, 1H, J = 8.0 Hz), 7.37–7.26 (m, 10H), 7.14 (dd, 1H, J = 1.2 Hz, J = 6.8 Hz), 6.98 (d, 2H, J = 8.8 Hz), 6.52 (s, 1H), 4.97 (s, 2H), 4.74 (s, 2H), 4.73 (s, 2H); MS (ESI), m/z: 550.10 [M−H]$^-$.

5.2.7.5. (E)-N-(3-Chlorophenyl)-2-(4-((2,5-dioxopyrrolidin-3-ylidene)methyl)phenoxy)acetamide ***7-54***. Procedure: **4a** (1.0 mmol), **8b** (1.0 mmol), and 10 mL MeOH were heated to reflux for 5 h. The mixture was filtered and washed by EtOH (2 × 10 mL), water (2 × 10 mL), and dried *in vacuum* at 40 °C for 24 h to afford **7-54**. Yield 23.4%; White powder; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.38 (s, 1H), 10.34 (s, 1H), 7.84 (s, 1H), 7.60 (d, 2H, J = 8.8 Hz), 7.54 (d, 1H, J = 8.0 Hz), 7.38–7.34 (m, 2H), 7.15 (d, 1H, J = 8.0 Hz), 7.08 (d, 2H, J = 8.8 Hz), 4.80 (s, 2H), 3.61 (s, 2H); MS (ESI), m/z: 369.02 [M−H]$^-$.

5.2.7.6. N-(3-Chlorophenyl)-2-(4-((2,4,6-trioxotetrahydropyrimidin-5(2H)-ylidene)methyl)phenoxy)acetamide ***7-55***. Procedure: aldehyde **4a** (0.5 mmol), ethanol (3 mL), distilled water (3 mL), and barbituric acid (3.0 mmol) were refluxed at 60 °C for 3–4 h. The formed solids were collected by filtration and washed with boiling water (3 × 15 mL), ethanol (3 × 15 mL), and ether (3 × 15 mL). The obtained solids were dried *in vacuo* for 24 h. Yield 43.9%; Yellow powder; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.33 (s, 1H), 11.20 (s, 1H), 10.37 (s, 1H), 8.36 (d, 2H, J = 8.8 Hz), 8.25 (s, 1H), 7.83 (t, 1H, J = 1.8 Hz), 7.54–7.51 (m, 1H), 7.37 (t, 1H, J = 8.4 Hz), 7.17–7.14 (m, 1H), 7.11 (d, 2H, J = 9.2 Hz), 4.88 (s, 2H); MS (ESI), m/z: 430.13 [M+MeOH−H]$^-$.

5.2.7.7. N-(3-Chlorophenyl)-2-(4-((4,6-dioxo-2-thioxotetrahydropyrimidin-5(2H)-ylidene)methyl)phenoxy)acetamide ***7-56***. Prepared by procedure for the synthesis of **7-55** using thiobarbituric acid. Yield 49.9%; Yellow powder; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.41 (s, 1H), 12.31 (s, 1H), 10.38 (s, 1H), 8.41 (d, 2H, J = 9.2 Hz), 8.27 (s, 1H), 7.83 (t, 1H, J = 2.0 Hz), 7.53 (d, 1H, J = 9.2 Hz), 7.37 (t, 1H, J = 8.0 Hz), 7.17–7.12 (m, 3H), 4.90 (s, 2H); MS (ESI), m/z: 446.09 [M+MeOH−H]$^-$.

5.2.7.8. (Z)-N-(3-Chlorophenyl)-2-(4-((2-imino-4-oxothiazolidin-5-ylidene)methyl)phenoxy)acetamide ***7-57***. Procedure: aldehyde **4a** (1.0 mmol), 2-thioxothiazolidin-4-one (1.2 mmol), piperidine (cat.), and 10 mL EtOH were mixed and heated to reflux for 6 h. Then a portion of water/ice was added and the precipitated solids were collected by sucking filtration and washed with distilled water (4 × 15 mL), EtOH (2 × 10 mL), and ether (2 × 10 mL). The solids obtained were dried in vacuum at 40 °C for 24 h. Yield 30.0%; Yellow powder; ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.80 (s, 1H), 10.358 (s, 1H), 7.84 (s, 1H), 7.62–7.59 (m, 3H), 7.53 (d, 1H, J = 8.4 Hz), 7.37 (t, 1H, J = 8.0 Hz), 7.18–7.15 (m, 3H), 4.84 (s, 2H); MS (ESI), m/z:

402.98 $[M-H]^-$.

5.2.7.9. (Z)-2-(5-(4-(2-((3-Chlorophenyl)amino)-2-oxoethoxy)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid ***7-58***. Prepared by procedure for the synthesis of **7-57** using 2-(4-oxo-2-thioxothiazolidin-3-yl)acetic acid. Yield 25.4%; Yellow powder; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.62 (s, 1H), 9.05 (br-s, 1H), 7.85 (s, 1H), 7.76 (s, 1H), 7.62 (d, 2H, J = 8.0 Hz), 7.55 (d, 1H, J = 8.0 Hz), 7.35 (t, 1H, J = 8.0 Hz), 7.17–7.13 (m, 3H), 4.85 (s, 2H), 4.42 (s, 2H); MS (ESI), m/z: 461.00 $[M-H]^-$.

5.2.7.10. (Z)-N-(3-Chlorophenyl)-2-(4-((2-imino-4-oxothiazolidin-5-ylidene)methyl)phenoxy)acetamide ***7-59***. Prepared by procedure for the synthesis of **7**–**57** using 2-iminothiazolidin-4-one. Yield 35.3%; Yellow powder; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.34 (s, 1H), 9.38 (s, 1H), 9.13 (s, 1H), 7.84 (s, 1H), 7.62–7.52 (m, 4H), 7.36 (t, 1H, J = 8.0 Hz), 7.15–7.13 (m, 3H), 4.81 (s, 2H); MS (ESI), m/z: 386.02 $[M-H]^-$.

5.3. Molecular docking assay

The iNOS was chosen as the target receptor. The 3D structure of the receptor was gained from Protein Data Bank (PDB ID: 1r35). Conformers of molecules were created by the aid of Omega and the up limit of conformer number for each molecule is set to 2000. Compound **7-44** was docked to the active site of iNOS by employing a protein-ligand docking program FRED. Scoring functions Chemgauss version two, shapegauss and screenscore were used for exhaustive searching, solid body optimizing and interaction scoring. This Fig. 3 was generated by the software Virtual Molecular Dynamics (VMD).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.12.036.

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