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Original article

Synthesis and biological activity of novel barbituric and thiobarbituric acid derivatives against non-alcoholic fatty liver disease

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ABSTRACT

Forty-four barbituric acid or thiobarbituric acid derivatives were synthesized and evaluated for their effects on adipogenesis of 3T3-L1 adipocytes by measuring the expression of adiponectin *in vitro*. Four compounds (**3a**, **3o**, **3s**, **4t**) were found to increase the expression of adiponectin and lower the leptin level in 3T3-L1 adipocytes at respective concentration of 10 μM. Among them, **3s** showed the most efficacious. Oral administration of **3s** effectively reduced body weight, liver weight, and visceral fat and regulated serum levels of biochemical markers in the high-fat/diet-induced Wistar rats. Histopathological evaluation of liver sections by Oil Red O and H&E staining confirmed **3s** as a potent, orally active molecule for reducing fat deposition against non-alcoholic fatty liver disease.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD), defined as fatty infiltration of the liver exceeding 5%–10% by weight, is a clinic pathological syndrome characterized by hepatic steatosis without excess alcohol intake. It has become one of the leading causes of chronic liver disease in many countries [1–3]. NAFLD comprises a wide spectrum ranging from simple fatty liver to non-alcoholic steatohepatitis (NASH), followed by progression to fibrosis, cirrhosis and hepatocellular carcinoma [4,5]. This disease is closely associated with insulin resistance (IR) and metabolic syndromes including obesity, type II diabetes, dyslipidemia, hypertension and atherosclerosis [6–9].

The adipose tissue secretes many physiologically active adipocytokines such as adiponectin and leptin, which modulate hepatic and peripheral lipid and glucose metabolism [10,11]. Adiponectin is a 30-kDa adipocyte protein exclusively expressed in adipose tissue of normal humans [12]. The plasma adiponectin level, directly associated with insulin sensitivity, is negatively correlated with body mass index (BMI), plasma glucose, insulin and triglycerides but positively correlated with the plasma levels of high-density lipoprotein (HDL)-cholesterol [13–15]. Leptin, the product of the obese gene, is an adipocyte-secreted protein hormone with an important role in energy homeostasis, including appetite, body weight and metabolism functions. The amount of leptin secreted and expressed by adipocytes is positively correlated with the lipid content and adipocyte size [16]. Therefore, the roles of adiponectin and leptin on regulating energy balance and metabolism make them as potential therapeutic implications for humans and animals of NAFLD.

As insulin resistance is very common in patients with non-alcoholic fatty liver disease, considerable studies developed novel insulin sensitizers as logical treatment strategies. Biguanides and thiazolidinediones (TZDs) are representatives insulin sensitizers widely used in clinic (Fig. 1). Metformin, whose mechanisms of action are not well understood, effectively improves hepatic steatosis in animal model of fatty liver [17] and aminotransaminase levels in human liver tissue [18] and increases insulin sensitivity by facilitating glucose consumption and utilization [19]. TZDs, such as rosiglitazone, are a kind of oral anti-diabetic medications that significantly ameliorate insulin resistance by acting as selective peroxisome proliferitor-activated receptor γ (PPAR γ) agonists [20]. In addition, TZDs increase circulating levels of adiponectin in adipose tissue [21–23] and has insulin sensitizing properties to slow

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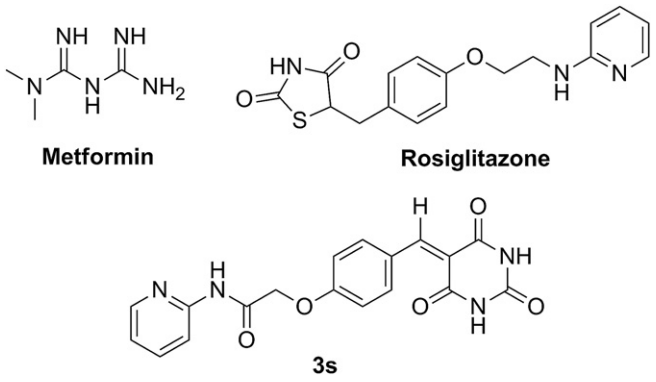


Fig. 1. Structure of Metformin, Rosiglitazone and **3s**.

and prevent the progression of NAFLD [24,25]. Recently, a new series of PPAR γ ligands based on barbituric acid instead of TZD were designed and evaluated and six active compounds were found to be bound to the murine PPAR γ with IC_{50} ranging from 0.1 to 2.5 μM [26]. Their results provided the impetus to develop novel and potent therapeutic agents containing barbituric acid or thiobarbituric acid moiety and investigate their pharmacological functions.

2. Chemistry

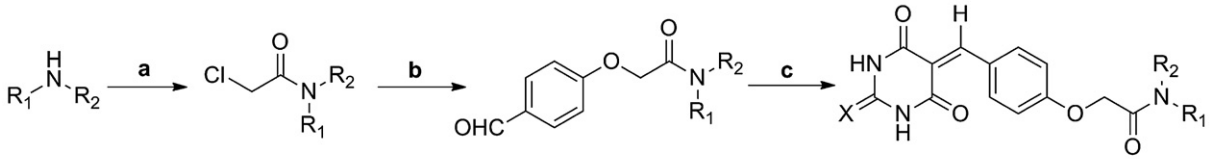
Various amines in this study were converted into chloroacetamide derivatives (**1a**–**v**) with excellent yields via a reaction employed 2-chloroacetyl chloride agent in the presence of triethylamine as base and dichloromethane as solvent. The similar reactions with the high yield were dangerous and toxic to organic synthetic operators because amount of hydrogen chloride gas was released. Therefore, the agent of 2-chloroacetyl chloride must be added slowly in the ice-bath with the application of absorption solution for tail gas. The pivotal aldehyde intermediates which contained 2-(4-formyl-phenoxy)-*N*-substituted-phenylacetamide (**2a**–**p**) and special aldehyde intermediates (**2q**–**v**) were synthesized through the use of chloroacetamide derivatives, 4-hydroxybenzaldehyde, potassium carbonate as base and potassium iodide as catalyst according to Scheme 1, respectively. The reaction mixture was refluxed in a solvent of acetone for 24 h and monitored by the thin-layer chromatography.

And then, to obtain the forty-four targeted compounds varying with the western substituted group quickly, the Knoevenagel reaction which accomplished by condensation aldehyde intermediate with babituric acid or thiobarbituric acid and employed water and ethanol as solvent was performed in parallel using EYELA Personal Organic Synthesizer (Tokyo, Rikakikai) with a 12-well liquid-phase reaction block. Through 4 or 5 h' reaction, the obtained crude was precipitated in the solvent, filtered and washed with ethyl ether and water because the babituric acid or thiobarbituric acid was dissolved into ethyl ether. The advantages of the Knoevenagel synthetic protocol were generalized [27]: (a) all starting materials were soluble in the mixture of water and ethanol; (b) the reaction was clean and quick; (c) the insoluble targeted compound was precipitated in the course of the reaction, yielding essentially pure final compound (purities ≥97%) and requiring no further purification. At this stage, the products were fully analyzed and characterized by hydrogen nuclear magnetic resonance (^{1}H NMR), Mass spectrum (MS), and High Performance Liquid Chromatography (HPLC) before entering the biological tests.

3. Biological results and discussion

3.1. In vitro assay for adiponectin expression in 3T3-L1 adipocytes

In order to quickly discover the potential therapeutic agents, the expression of adiponectin was evaluated by measuring the promoting rate of the adiponection in cultured 3T3-L1 adipocytes. Rosiglitazone (Fig. 1) was selected as a positive control. Forty-four compounds based on barbituric acid and thiobarbituric acid moiety with respective concentration of 10 μM were investigated the effects on differentiation of 3T3-L1 preadipocytes into adipocytes by measuring the promoting rates of adiponectin expression. Their results were depicted in Fig. 2.



3,4 a:	R_1 = Ph,	R_2 = H	3,4 l:	R_1 = 2,4-diCl-Ph,	R_2 = H
3,4 b:	R_1 = 4-CH_3-Ph,	R_2 = H	3,4 m:	R_1 = 3,5-diCl-Ph,	R_2 = H
3,4 c:	R_1 = 4-OCH_3-Ph,	R_2 = H	3,4 n:	R_1 = 3-Cl,4-F-Ph,	R_2 = H
3,4 d:	R_1 = 3,4-diOCH_3-Ph,	R_2 = H	3,4 o:	R_1 = 3,4-diF-Ph,	R_2 = H
3,4 e:	R_1 = 4-CF_3-Ph,	R_2 = H	3,4 p:	R_1 = Ph,	R_2 = CH_3
3,4 f:	R_1 = 4-CH_3C(O)-Ph,	R_2 = H	3,4 q:	R_1 = cyclohexyl,	R_2 = H
3,4 g:	R_1 = 3-F-Ph,	R_2 = H	3,4 r:	R_1 = benzyl,	R_2 = H
3,4 h:	R_1 = 4-F-Ph,	R_2 = H	3,4 s:	R_1 = pyridin-2-yl,	R_2 = H
3,4 i:	R_1 = 3-Cl-Ph,	R_2 = H	3,4 t:	R_1 = 5-methylpyridin-2-yl,	R_2 = H
3,4 j:	R_1 = 4-Cl-Ph,	R_2 = H	3,4 u:	R_1 = naphthalen-1-yl,	R_2 = H
3,4 k:	R_1 = 4-Br-Ph,	R_2 = H	3,4 v:	R_1 = naphthalen-2-yl,	R_2 = H

Scheme 1. General synthesis of **3**, **4a**–**v**. Reagents and conditions: (a) 2-chloroacetyl chloride, Et_3N, CH_2Cl_2, 0 °C–25 °C, 20 h; (b) KI, K_2CO_3, 4-hydroxybenzaldehyde, reflux, 24 h; (c) barbituric acid or thiobarbituric acid, ethanol, H_2O, 60 °C, 3–4 h.

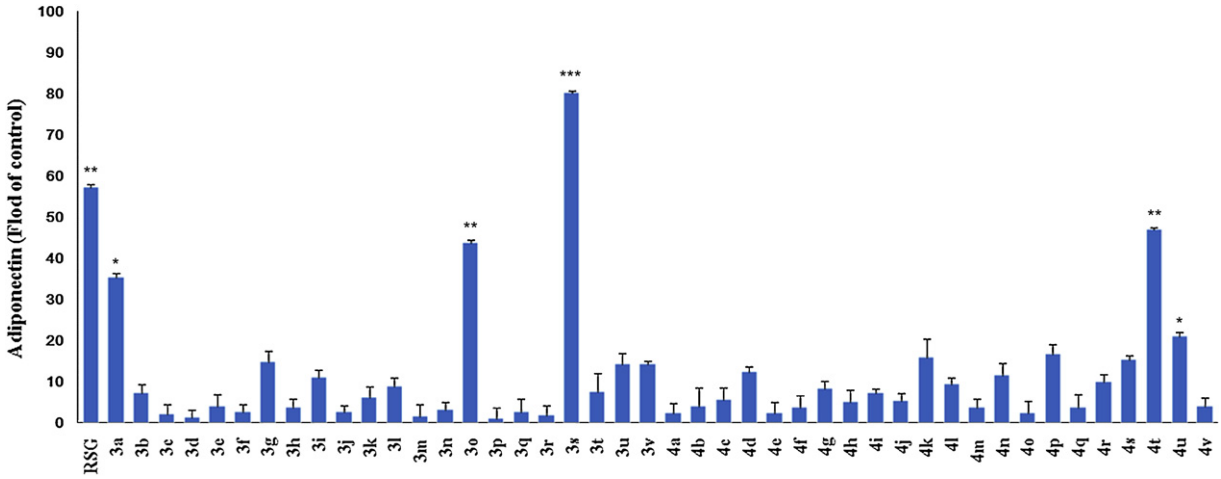


Fig. 2. Expression of adiponectin under the actions of **3**, **4a**–**v** in 3T3-L1 adipocytes. Rosiglitazone (RSG) and **3**, **4a**–**v** were at a same concentration of 10 μM. Results were at least three independent experiments. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ vs. vehicle (as baseline).

As shown in Fig. 2, the initial screening data obtained from the compounds indicated that promoting rates of adiponectin expression of compounds (**3a**, **3o**, **3s** and **4t**) were up-regulated by 135.18%, 143.80%, 180.32% and 146.94%, respectively (The promoting rate of vehicle was defined as a 100%). Among them, **3s** exhibited the most efficacious compared with positive compound of rosiglitazone which the promoting rate of adiponectin expression was 157.31%.

As for **3a**–**p** and **4a**–**p**, the substituents of electron-donating group (methyl, methoxyl and trifluromethyl group) and electron-withdrawing group (acetyl group, halogen including fluorine, chlorine and bromine atom) were introduced in the western substituted part and these compounds were observed no or much lower adiponectin expressions, with the only exception being the 3, 4-difluro group which significantly promoted the expression of adiponectin. Additionally, varying with cyclohexyl, benzyl and naphthyl group in the western group was no remarkable increment of adiponectin expression. By inspection, one explanation about the strong activities of **3s** and **4t** argued that the pyridine group possessed perfect hydrophilic property in comparison to the remaining compounds whose western parts were all hydrophobic groups.

3.2. Expression of adiponectin and leptin in 3T3-L1 adipocytes in vitro

In order to evaluate the effects of **3a**, **3o**, **3s** and **4t** on adipogenesis of 3T3-L1 adipocytes, we further determined the expression of adiponectin and leptin in 3T3-L1 adipocytes by commercial kits (Table 1). Consistent with the results obtained from promoting rates of adiponectin expression at a concentration of 10 μM, the four compounds significantly down-regulated the expression of leptin in contrast to rosiglitazone and the **3s** was the most potent, worthy of further biological assays as a potential agent for non-alcoholic fatty liver disease. Additionally, the four compounds were no distinct cytotoxicity compared to vehicle in 3T3-L1 adipocytes (S2 in Supplementary data).

3.3. Measurement of biochemical markers in treated and untreated Wistar rat model with NAFLD

Further *in vivo* studies in male Wistar rats with NAFLD induced by high-fat/high calorie diet (HF/HC) confirmed our *in vitro* screening results. After oral administration of **3s** once a day at a dose of 50 mg/kg for four weeks, the average decrease in body weight, liver weight and visceral fat reached respectively 16.4%, 25.3% and 19.63% compared with the untreated HF/HC diet group and further the **3s**-treated rats were nearly equal-weight to those of the normal group, as depicted in Table 2. Throughout the *in vivo* experiment, decrements in levels of FBS, TG and ALT were 48.77%, 57.23% and 30.43%, respectively. The insulin sensitivity was assessed by HOMA-IR values and increased significantly through treatment with **3s**, as indicated by a 54.08% reduction in HOMA-IR. Consistent with our *in vitro* results, the serum levels of adiponectin and leptin had achieved to normal levels. The levels of low-density lipoprotein (LDL)-cholesterol were down-regulated from 0.97 mM in HF/HC diet model to 0.175 mM in **3s**-treated model. However, the serum levels of high-density lipoprotein (HDL)-cholesterol were unexpected to ascend after induced by HF/HC diet. The possible reason was that the amount of cholesterol was elevated in serum of Wistar rats access to HF/HC diet, and then the HDL tended to carry cholesterol away from the arteries and back to the liver for excretion or reutilization. Nevertheless, no abnormal behavioral or physiological signs were observed during the experiment. In general, **3s** significantly alleviated the progression of NAFLD in male Wistar rats.

3.4. Histopathological evaluation

To evaluate whether **3s** improved fat deposition and recruitment of liver cells in Wistar rat model with NAFLD, liver tissues from **3s**-treated and vehicle-treated rats were processed routinely for Oil Red O and H&E staining assay (Fig. 3). As shown in Oil Red O staining, we found the distinctly increased number of fat deposition (the red sections) in the liver tissue from Wistar rats access to HF/HC diet versus the normal ones, whereas administration of **3s** decreased efficaciously the fat accumulation of liver sections.

Table 1
Expression values of adiponectin and leptin in 3T3-L1 adipocytes.

Compound [a]	Adiponectin (ng/mL)	Leptin (pg/mL)
Vehicle	843.78 ± 20.61	3089.21 ± 33.37
RSG	1324.73 ± 42.47 **	1773.67 ± 182.89 **
3a	1111.73 ± 91.60	1856.32 ± 158.25*
3o	1322.02 ± 62.84 *	1571.98 ± 95.13
3s	1639.61 ± 30.25 **	1271.98 ± 40.75 **
4t	1239.44 ± 74.05*	1514.81 ± 80.95

$^{*}P < 0.05$; $^{**}P < 0.01$ vs. vehicle.
[a] Gosiglitazone (RSG) and **3a**, **3o**, **3s** and **4t** were at a concentration of 10 μM. Results were means ± SD of at least three independent experiments.

Table 2
Biochemical characteristics of untreated and **3s**-treated NAFLD rat model access to HF/HC diet for 10 weeks.

Parameters	Normal	HF/HC	HF/HC+3s[a]
Body weight (g)	349.8 ± 15.9	431.0 ± 19.4	360.5 ± 11.9*
Liver weight (g)	15.0 ± 2.7	21.4 ± 3.1	16.0 ± 2.0
Liver/body weight (%)	4.29	4.95	4.34*
Visceral fat (g/100g BW)	3.84 ± 0.31	4.95 ± 0.95	3.98 ± 0.39
FBS (mM)	4.77 ± 0.42	9.23 ± 1.68	4.73 ± 0.86**
Insulin (μU/mL)	29.03 ± 2.68	36.32 ± 2.97	33.43 ± 2.11*
HOMA-IR[b]	6.15 ± 0.05	15.31 ± 0.22	7.03 ± 0.08**
TG (mM)	1.18 ± 0.12	2.38 ± 1.20	1.02 ± 0.98*
ALT (U/L)	30.12 ± 3.48	47.52 ± 4.98	33.06 ± 2.69**
Adiponectin (μg/mL)	12.87 ± 2.50	10.49 ± 2.59	12.53 ± 1.27*
Leptin (ng/mL)	5.77 ± 1.84	7.59 ± 2.36	6.00 ± 1.01*
LDL-cholesterol (mM)	0.44 ± 0.23	0.97 ± 0.31	0.18 ± 0.09*
HDL-cholesterol (mM)	0.69 ± 0.12	1.20 ± 0.43	1.11 ± 0.91

*$P < 0.05$; **$P < 0.01$ vs. normal.
[a] **3s** was orally administrated once a day at a dose of 50 mg/kg for four weeks. Results were means ± SD.
[b] HOMA-IR [31] was defined as follows. HOMA-IR = [FBS (mM) × Insulin (μU/mL)]/22.5.

In addition, fat deposition ruined severely the liver histology presenting as ballooning degeneration, and numerous lipid droplets as evidenced by H&E staining. After the rats were treated with **3s**, recruitment and restoration of liver cells versus the normal ones were improved remarkably. The results suggested that **3s** reduced fat deposition effectively and possessed liver protective effects with non-alcoholic fatty liver disease.

4. Conclusion

In this present study, we discovered a novel compound **3s** and investigated its pharmacological effects as a potential therapeutic agent for the treatment of HF/HC diet-induced non-alcoholic fatty liver disease in male Wistar rats. In addition, we adopted a feasible and credible drug screening method on adiponectin expression for our forty-four synthetic compounds *in vitro*, and then followed by serum assays of ALT, TG, FBS, insulin, adiponectin, leptin, LDL-cholesterol and histopathological evaluation *in vivo*. Our results indicated that **3s** based on barbituric acid at a concentration of 10 μM significantly up-regulated the expression of adiponectin as well as down-regulated the expression of leptin in 3T3-L1 adipocytes and further promoted glucose consumption with insulin (0.1 μM) in HepG2 cell lines (see S3 in supplementary data), which exceeded the potency of known insulin sensitizers, rosiglitazone and metformin. Serum assay and histopathological evaluation further validated that **3s** remarkably reduced lipid accumulation in the liver tissue correlated with NAFLD. However, additional studies are still warranted on the pharmacology of the described compounds for an evaluation of their mechanism and pharmacokinetic properties and for a deeper investigation of their activity profile against NAFLD.

5. Experimental section

5.1. Biological methods

5.1.1. 3T3-L1 Adipocytes differentiation

Marine 3T3-L1 preadipocytes (American Type Culture Collection, Rockville, MD) were plated and grown to two days post-confluence in 96 or 6 well culture plates in DMEM containing 10% fetal bovine serum; Preadipocytes were induced to differentiate by replacing the medium with serum-containing DMEM containing 0.5 mM methyl-3-isobutylxantine (IBMX), 0.25 μM dexamethasone (DEX) and 1 μg/mL insulin. Two days later, the medium was again

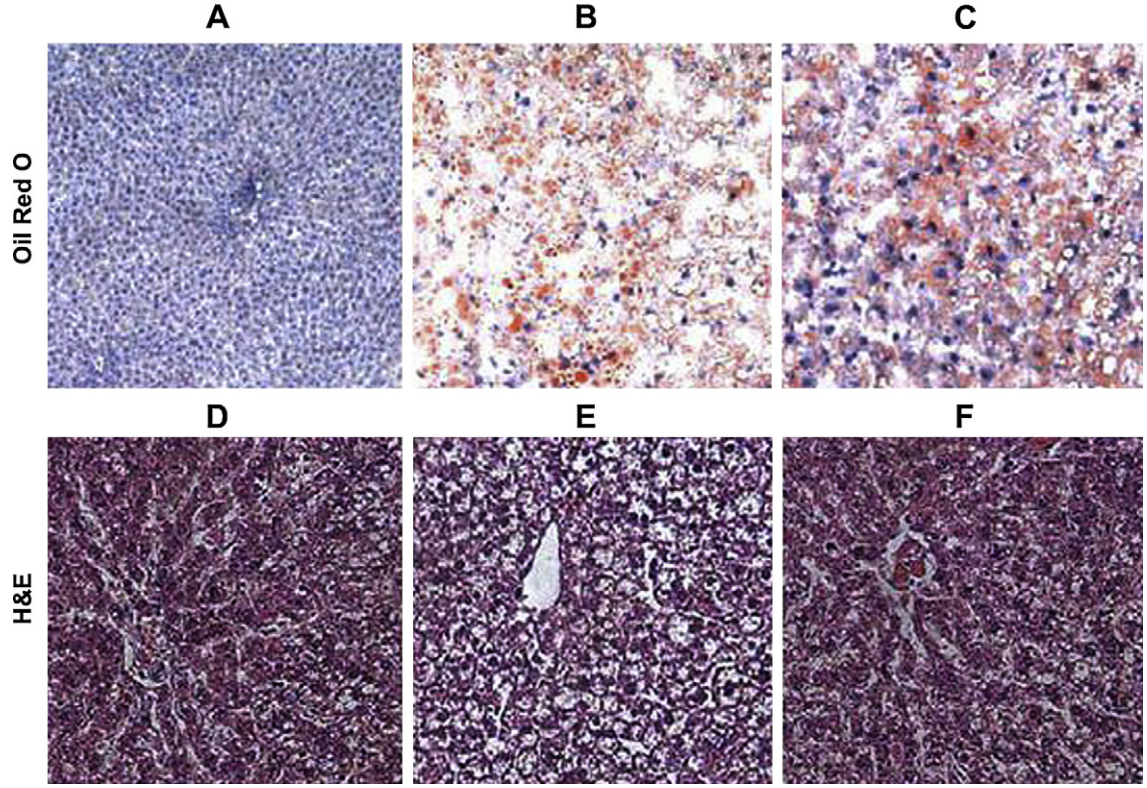


Fig. 3. Oil Red O and H&E staining of liver sections of untreated and 3s-treated NAFLD rat model access to HF/HC diet. Oil Red O staining: (A) Normal diet; (B) Model access to HF/HC diet; (C) 3s-treated model access to HF/HC diet. H&E staining: (D) Normal diet; (E) Model access to HF/HC diet; (F) 3s-treated model access to HF/HC diet.

changed to serum-containing DMEM that contained insulin but no IBMX or DEX. Two days later, the medium was again changed to the original DMEM containing 10% fetal bovine serum in the absence of any differentiating reagents and was replaced every two days. Full differentiation is usually achieved by 8–12 days.

5.1.2. Expression levels of adiponectin and leptin in 3T3-L1 adipocytes

The twelfth day after differentiation, the culture medium was replaced by DMEM supplemented with chemical series of **3** and **4** (10 μM) and rosiglitazone (Sigma–Aldrich, St. Louis, MO, USA) (10 μM). After 24 h, the expression levels of adiponectin and leptin were measured by commercial kits (Linco Research, St. Charles, MO, USA).

5.1.3. Animal model and treatment of NAFLD

Normal male Wistar rats weighing 140–180 g were purchased from Western China Experimental Animal Center. Every five rats were placed in one cage on a 12 h day/night cycle and quarantined for 1 week. All animals, which were maintained under controlled conditions and had free access to standard laboratory chow and water, received human care according to National Institutes of Health Guidelines before and during experiment. After acclimatization for 1 week, animals were assigned to four groups randomly consisting of 10 rats each (G1–G3). In this study, G1–G2 groups were given access to high-fat diet and G3 group access to normal diet throughout the experimental period, respectively. The normal diet was purchased from Shuangshi experimental animal diet center (Jiangshu province, PR China). The high-fat/high calorie diet consisted of 20% lard stearin (wt/wt), 10% sucrose and 0.1% bile salt were added into normal diet. The rats received a normal diet with 18.94% of energy derived from fat, 31.67% from protein and 49.39% from carbohydrates, which received a high-fat diet with 50.55% of energy derived from fat, 15.72% from protein and 33.73% from carbohydrates. In addition to the daily diet, compound **3s** suspended in Tween 80 and 5% saline (Sigma–Aldrich, St. Louis, MO), were administered correspondingly to G1 group orally once a day at a dose of 50 mg/kg. After the experimental period completed, all rats were anesthetized and samples were collected. Meanwhile, body weight and liver weight of rats was recorded.

5.1.4. Assay for serum biochemical markers

Serum ALT levels were measured by an automated enzyme assay using commercial kits and Roche automated analyzers (Roche Diagnostics GmbH, Manheim, Germany). Serum levels of TG, LDL-cholesterol, HDL-cholesterol, FBS and plasma insulin were determined by radioimmunoassay using commercially available kits (Linco Research, St. Charles, MO, USA).

5.1.5. Histopathological examination

Liver samples were fixed in 4% buffered formalin and embedded in Tissue-Tek OCT compound (Sakura Finetek USA, California, USA) and paraffin for histological analysis. Formalin-fixed and paraffin-embedded section (5 μm) was processed routinely for H&E staining. The OCT-embedded samples were serially sectioned at 4 μm. For the evaluation of fat deposition, the liver section was stained with Oil red O.

5.2. Chemistry

Chemical reagents of analytical grade were purchased from Chengdu Changzheng Chemical Factory (Sichuan, PR China). The final compounds were synthesized using an EYELA Personal Organic Synthesizer with ChemiStation PPS-CTRL and PPW-20A (Tokyo, Rikakikai) using a 5-well liquid-phase reaction block. TLC was performed on 0.20 mm silical gel 60 F_{254} plates (Qingdao Ocean Chemical Factory, Shangdong, China). The purity of compound screened in biological assays was determined to be ≥97% by HPLC analysis with a phodediode array detector (Waters, Milford, MA, USA) and the chromatographic column was an atlantis C_{18} (150 mm × 4.6 mm, i.d. 5 μm) (Waters, Milford, Ireland). Hydrogen Nuclear magnetic resonance spectra (^{1}H NMR) were recorded at 400 MHz on a Varian spectrometer (Varian, Palo Alto, CA, USA) model Gemini 400 and reported in parts per million. Chemical shifts (δ) are quoted in ppm relative to tetramethylsilane (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 (MS) were measured by Q-TOF Priemier mass spectrometer utilizing electrospray ionization (ESI) (Micromass, Manchester, UK). Room temperature (RT) is within the range 20–25 °C.

*5.2.1. General procedure for synthesis of 2-chloro-N-substituted-acetamide (**1a–v**)*

2-Chloroacetyl chloride (24 mmol) was slowly added dropwise to a mixture of R-NH_2 (20 mmol) and Et_3N (24 mmol, 3.3 mL) in anhydrous CH_2Cl_2 (20 mL) at 0 °C. The reaction mixture was warmed to room temperature and stirred for an additional 20 h. After the solvent was removed under reduced pressure, the residue was washed with ice water (3 × 20 mL) and the precipitate was separated by filtration. The crude product was purified by crystallization from a mixture solvent of Et_2O/petroleum.

*5.2.2. General procedure for synthesis of 2-(4-formyl-phenoxy)-N-substituted-phenyl-acetamide and special aldehyde intermediates (**2a–v**)*

4-hydroxybenzaldehyde (1.34 g, 11 mmol), anhydrous K_2CO_3 (2.76 g, 20 mmol) and the 2-chloro-*N*-substituted-acetamide (10 mmol) were dissolved in anhydrous acetone (30 mL), and then KI (166 mg, 1 mmol) were added into the solution. The reaction mixture was refluxed for 24 h 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 (eluent: ethyl acetate/petroleum = 1/1.5) to give the appropriate aldehyde product.

*5.2.3. General procedure for synthesis of **3**, **4a–v***

The appropriate aldehyde (3.5 mmol), ethanol (10 mL), distilled water (10 mL), and (thio) barbituric acid (3 mmol) were added in parallel in 12 test tubes with reflux condensers and stirred at 1000 rpm in tubes at 80 °C for 3–4 h. The formed solids were collected by sucking filtration and washed with boiling water (3 × 50 mL), ethanol (3 × 50 mL), and ether (3 × 25 mL). The colorful solids obtained were dried in vacuum at 40 °C for 12 h.

*5.2.3.1. N-Phenyl-2-(4-((2,4,6-trioxotetrahydropyrimidin-5(6H)-ylidene)methyl)phenoxy)acetamide (**3a**).* Yield: 89.4%; HPLC: 99.6%; Yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.32 (s, 1H), 11.19 (s, 1H), 10.17 (s, 1H), 8.36 (d, 2H, J = 8.8 Hz), 8.25 (s, 1H), 7.63 (d, 2H, J = 7.6 Hz), 7.33 (t, 2H, J = 8.0 Hz), 7.13–7.07 (m, 2H), 4.86 (s, 2H); ^{13}C NMR (400 MHz, DMSO-d_6): δ 167.69, 162.10, 161.89, 157.31, 154.54, 150.42, 138.14, 130.28, 130.20, 128.49, 128.68, 121.56, 121.60, 119.12, 114.20, 114.15, 65.89; MS (ESI), m/z: 364.18 $[M - H]^-$.

*5.2.3.2. N-p-Tolyl-2-(4-((2,4,6-trioxotetrahydropyrimidin-5(6H)-ylidene)methyl)phenoxy)acetamide (**3b**).* Yield: 78.0%; HPLC: 98.4%; Yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.32 (s, 1H), 11.19 (s, 1H), 10.08 (s, 1H), 8.36 (d, 2H, J = 9.2 Hz), 8.25 (s, 1H), 7.51 (d, 2H,

J = 8.4 Hz), 7.12 (t, 4H, J = 8.0 Hz), 4.84 (s, 2H), 2.26 (s, 3H); MS (ESI), m/z: 378.15 [M − H]$^-$.

*5.2.3.3. N-(4-Methoxyphenyl)-2-(4-((2,4,6-trioxotetrahydropyrimidin-5(6H)-ylidene)methyl)phenoxy)acetamide (**3c**).* Yield: 89.4%; HPLC: 98.5%; Yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.32 (s, 1H), 11.19 (s, 1H), 10.03 (s, 1H), 8.36 (d, 2H, J = 10.2 Hz), 8.25 (s, 1H), 7.54 (d, 2H, J = 10.2 Hz), 7.11 (d, 2H, J = 8.4 Hz), 6.90 (d, 2H, J = 7.2 Hz), 4.82 (s, 2H), 3.73 (s, 3H); MS (ESI), m/z: 394.20 [M − H]$^-$.

*5.2.3.4. N-(3,4-Dimethoxyphenyl)-2-(4-((2,4,6-trioxotetrahydropyrimidin-5(6H)-ylidene)methyl)phenoxy)acetamide (**3d**).* Yield: 76.0%; HPLC: 99.8%; Yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.32 (s, 1H), 11.19 (s, 1H), 10.02 (s, 1H), 8.37 (d, 2H, J = 8.8 Hz), 8.26 (s, 1H), 7.36 (d, 1H, J = 2.0 Hz), 7.17−7.10 (m, 3H), 6.91 (d, 1H, J = 8.8 Hz), 4.83 (s, 2H), 3.72 (s, 6H); MS (ESI), m/z: 424.07 [M − H]$^-$.

*5.2.3.5. N-(4-(Trifluoromethyl)phenyl)-2-(4-((2,4,6-trioxotetrahydropyrimidin-5(6H)-ylidene)methyl)phenoxy)acetamide (**3e**).* Yield: 83.1%; HPLC: 99.5%; Yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.32 (s, 1H), 11.19 (s, 1H), 10.54 (s, 1H), 8.35 (d, 2H, J = 8.8 Hz), 8.25 (s, 1H), 7.85 (d, 2H, J = 8.0 Hz), 7.70 (d, 2H, J = 8.4 Hz), 7.11 (d, 2H, J = 8.8 Hz), 4.91 (s, 2H); MS (ESI), m/z: 464.08 [M − MeOH]$^-$.

*5.2.3.6. N-(4-Acetylphenyl)-2-(4-((2,4,6-trioxotetrahydropyrimidin-5(6H)-ylidene)methyl)phenoxy)acetamide (**3f**).* Yield: 97.5%; HPLC: 99.4%; Yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.32 (s, 1H), 11.19 (s, 1H), 10.52 (s, 1H), 8.35 (d, 2H, J = 8.8 Hz), 8.25 (s, 1H), 7.95 (d, 2H, J = 8.4 Hz), 7.77 (d, 2H, J = 8.8 Hz), 7.11 (d, 2H, J = 9.2 Hz), 4.91 (s, 2H), 2.53 (s, 3H); MS (ESI), m/z: 438.26 [M − MeOH]$^-$.

*5.2.3.7. N-(3-Fluorophenyl)-2-(4-((2,4,6-trioxotetrahydropyrimidin-5(6H)-ylidene)methyl)phenoxy)acetamide (**3g**).* Yield: 88.7%; HPLC: 97.3%; Light yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.31 (s, 1H), 11.19 (s, 1H), 10.38 (s, 1H), 8.35 (d, 2H, J = 8.8 Hz), 8.25 (s, 1H), 7.60 (d, 1H, J = 11.6 Hz), 7.37−7.35 (m, 2H), 7.10 (d, 2H, J = 8.8 Hz), 6.92−6.90 (m, 1H), 4.87 (s, 2H); MS (ESI), m/z: 414.11 [M − MeOH]$^-$.

*5.2.3.8. N-(4-Fluorophenyl)-2-(4-((2,4,6-trioxotetrahydropyrimidin-5(6H)-ylidene)methyl)phenoxy)acetamide (**3h**).* Yield: 86.1%; HPLC: 99.4%; Yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.32 (s, 1H), 11.19 (s, 1H), 10.23 (s, 1H), 8.36 (d, 2H, J = 9.2 Hz), 8.25 (s, 1H), 7.67−7.63 (m, 2H), 7.18 (t, 2H, J = 8.8 Hz), 7.11 (d, 2H, J = 8.0 Hz), 4.86 (s, 2H); MS (ESI), m/z: 414.19 [M − MeOH]$^-$.

*5.2.3.9. N-(3-Chlorophenyl)-2-(4-((2,4,6-trioxotetrahydropyrimidin-5(6H)-ylidene)methyl)phenoxy)acetamide (**3i**).* Yield: 95.0%; HPLC: 99.8%; Light yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.31 (s, 1H), 11.19 (s, 1H), 10.35 (s, 1H), 8.35 (d, 2H, J = 8.8 Hz), 8.25 (s, 1H), 7.83 (s, 1H), 7.52 (d, 1H, J = 8.0 Hz), 7.36 (t, 1H, J = 8.0 Hz), 7.15−7.10 (m, 3H), 4.87 (s, 2H); MS (ESI), m/z: 430.13 [M − MeOH]$^-$.

*5.2.3.10. N-(4-Chlorophenyl)-2-(4-((2,4,6-trioxotetrahydropyrimidin-5(6H)-ylidene)methyl)phenoxy)acetamide (**3j**).* Yield: 45.0%; HPLC: 99.2%; Yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.32 (s, 1H), 11.19 (s, 1H), 10.32 (s, 1H), 8.36 (d, 2H, J = 8.8 Hz), 8.25 (s, 1H), 7.67 (d, 2H, J = 8.4 Hz), 7.39 (d, 2H, J = 8.8 Hz), 7.11 (d, 2H, J = 8.8 Hz), 4.87 (s, 2H); MS (ESI), m/z: 398.11 [M − H]$^-$.

*5.2.3.11. N-(4-Bromophenyl)-2-(4-((2,4,6-trioxotetrahydropyrimidin-5(6H)-ylidene)methyl)phenoxy)acetamide (**3k**).* Yield: 95.6%; HPLC: 98.4%; Yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.32 (s, 1H), 11.19 (s, 1H), 10.31 (s, 1H), 8.35 (d, 2H, J = 8.8 Hz), 8.24 (s, 1H), 7.61 (d, 2H, J = 8.8 Hz), 7.51 (s, 2H, J = 8.8 Hz), 7.10 (d, 2H, J = 8.8 Hz), 4.86 (s, 2H); MS (ESI), m/z: 442.02 [M − H]$^-$.

*5.2.3.12. N-(2,4-Dichlorophenyl)-2-(4-((2,4,6-trioxotetrahydropyrimidin-5(6H)-ylidene)methyl)phenoxy)acetamide (**3l**).* Yield: 87.3%; HPLC: 99.6%; Light yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.33 (s, 1H), 11.20 (s, 1H), 9.84 (s, 1H), 8.36 (d, 2H, J = 8.8 Hz), 8.26 (s, 1H), 7.81 (d, 1H, J = 8.4 Hz), 7.47−7.44 (m, 1H), 7.17−6.98 (m, 1H), 7.13 (d, 2H, J = 8.8 Hz), 4.95 (s, 2H); MS (ESI), m/z: 464.19 [M − MeOH]$^-$.

*5.2.3.13. N-(3,5-Dichlorophenyl)-2-(4-((2,4,6-trioxotetrahydropyrimidin-5(6H)-ylidene)methyl)phenoxy)acetamide (**3m**).* Yield: 92.1%; HPLC: 99.7%; Yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.33 (s, 1H), 11.20 (s, 1H), 10.49 (s, 1H), 8.36 (d, 2H, J = 8.8 Hz), 8.26 (s, 1H), 7.74 (d, 2H, J = 1.6 Hz), 7.33 (s, 1H), 7.12 (d, 2H, J = 9.2 Hz), 4.90 (s, 2H); MS (ESI), m/z: 465.94 [M − MeOH]$^-$.

*5.2.3.14. N-(3-Chloro-4-fluorophenyl)-2-(4-((2,4,6-trioxotetrahydropyrimidin-5(6H)-ylidene)methyl)phenoxy)acetamide (**3n**).* Yield: 98.7%; HPLC: 99. 6%; Yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.32 (s, 1H), 11.19 (s, 1H), 10.38 (s, 1H), 8.35 (d, 2H, J = 8.8 Hz), 8.25 (s, 1H), 7.95−7.93 (m, 1H), 7.58−7.54 (m, 1H), 7.40 (d, 1H, J = 8.8 Hz), 7.11 (d, 2H, J = 8.8 Hz), 4.87 (s, 2H); MS (ESI), m/z: 416.09 [M − H]$^-$.

*5.2.3.15. N-(3,4-Difluorophenyl)-2-(4-((2,4,6-trioxotetrahydropyrimidin-5(6H)-ylidene)methyl)phenoxy)acetamide (**3o**).* Yield: 94.4%; HPLC: 99.8%; Light yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.32 (s, 1H), 11.19 (s, 1H), 10.39 (s, 1H), 8.35 (d, 2H, J = 8.8 Hz), 8.25 (s, 1H), 7.82−7.77 (m, 1H), 7.47−7.38 (m, 2H), 7.11 (d, 2H, J = 8.4 Hz), 4.87 (s, 2H); ^{13}C NMR (400 MHz, DMSO-d_6): δ167.84, 162.33, 162.13, 157.38, 154.56, 150.42, 147.21, 145.11, 135.76, 130.23, 130.25, 125.65, 121.78, 118.59, 114.89, 114.75, 114.13, 111.70, 59.61; MS (ESI), m/z: 432.27 [M − MeOH]$^-$.

*5.2.3.16. N-Methyl-N-phenyl-2-(4-((2,4,6-trioxotetrahydropyrimidin-5(6H)-ylidene)methyl)phenoxy)acetamide (**3p**).* Yield: 83.8%; HPLC: 99.0%; Yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.30 (s, 1H), 11.17 (s, 1H), 8.30 (d, 2H, J = 8.4 Hz), 8.22 (s, 1H), 7.500−7.41 (m, 5H), 6.89 (s, 2H), 4.59 (s, 2H), 3.20 (s, 3H); MS (ESI), m/z: 410.28 [M − MeOH]$^-$.

*5.2.3.17. N-Cyclohexyl-2-(4-((2,4,6-trioxotetrahydropyrimidin-5(6H)-ylidene)methyl)phenoxy)acetamide (**3q**).* Yield: 87.8%; HPLC: 99.1%; Light yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.31 (s, 1H), 11.19 (s, 1H), 8.34 (d, 1H, J = 8.8 Hz), 8.25 (s, 1H), 7.98 (d, 1H, J = 8.0 Hz), 7.05 (d, 2H, J = 9.2 Hz), 4.60 (s, 2H), 3.61 (t, 1H, J = 4.4 Hz), 1.74−1.68 (m, 4H), 1.58−1.55 (m, 1H), 1.32−1.19 (m, 4H), 1.15−1.06 (m, 1H); MS (ESI), m/z: 402.14 [M − MeOH]$^-$.

*5.2.3.18. N-Benzyl-2-(4-((2,4,6-trioxotetrahydropyrimidin-5(6H)-ylidene)methyl)phenoxy)acetamide (**3r**).* Yield: 85.9%; HPLC: 99.5%; Light yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.31 (s, 1H), 11.19 (s, 1H), 8.73 (t, 1H, J = 6.0 Hz), 8.34 (d, 2H, J = 8.4 Hz), 8.25 (s, 1H), 7.33−7.21 (m, 5H), 7.08 (d, 2H, J = 8.4 Hz), 4.71 (s, 2H), 4.35 (d, 2H, J = 6.0 Hz); MS (ESI), m/z: 410.22 [M − MeOH]$^-$.

*5.2.3.19. N-(Pyridin-2-yl)-2-(4-((2,4,6-trioxotetrahydropyrimidin-5(6H)-ylidene)methyl)phenoxy)acetamide (**3s**).* Yield: 84.9%; HPLC: 99.1%; Light yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.32 (s, 1H), 11.19 (s, 1H), 10.66 (s, 1H), 8.35 (d, 3H, J = 8.8 Hz), 8.25 (s, 1H), 8.04 (d, 1H, J = 8.4 Hz), 7.83−7.78 (m, 1H), 7.16−7.13 (m, 1H), 7.09 (d, 2H, J = 8.4 Hz), 4.96 (s, 2H); ^{13}C NMR (400 MHz, DMSO-d_6): δ 169.48, 162.30, 162.15, 157.31, 154.69, 151.49, 150.41, 146.71, 138.74, 130.21, 130.24, 125.29, 124.49, 118.90, 115.78, 114.28, 114.21, 66.48; MS (ESI), m/z: 397.11 [M − MeOH]$^-$.

*5.2.3.20. N-(5-Methylpyridin-2-yl)-2-(4-((2,4,6-trioxotetrahydropyrimidin-5(6H)-ylidene)methyl)phenoxy)acetamide (**3t**).* Yield: 89.4%; HPLC: 98.7%; Light yellow solid; ^{1}H NMR (400 MHz,

DMSO-d_6) δ 11.32 (s, 1H), 11.19 (s, 1H), 10.56 (s, 1H), 8.35 (d, 2H, J = 8.8 Hz), 8.25 (s, 1H), 8.18 (s, 1H), 7.95 (d, 1H, J = 7.6 Hz), 7.63 (dd, 1H, J = 8.4 Hz), 7.08 (d, 2H, J = 9.2 Hz), 4.94 (s, 2H), 2.26 (s, 3H); MS (ESI), m/z: 411.13 [M − MeOH]$^-$.

*5.2.3.21. N-(Naphthalen-1-yl)-2-(4-((2,4,6-trioxotetrahydropyrimidin-5(6H)-ylidene)methyl)phenoxy)acetamide (**3u**).* Yield: 89.2%; HPLC: 99.1%; Light yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.32 (s, 1H), 11.20 (s, 1H), 10.23 (s, 1H), 8.39 (d, 2H, J = 8.8 Hz), 8.27 (s, 1H), 8.01–7.95 (m, 2H), 7.81 (d, 1H, J = 8.0 Hz), 7.67–7.65 (m, 1H), 7.55–7.49 (m, 3H), 7.18 (d, 2H, J = 8.0 Hz), 5.03 (s, 2H); MS (ESI), m/z: 446.16 [M − MeOH]$^-$.

*5.2.3.22. N-(Naphthalen-2-yl)-2-(4-((2,4,6-trioxotetrahydropyrimidin-5(6H)-ylidene)methyl)phenoxy)acetamide (**3v**).* Yield: 96.3%; HPLC: 99.5%; Light yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.33 (s, 1H), 11.20 (s, 1H), 10.40 (s, 1H), 8.37 (d, 2H, J = 8.8 Hz), 8.32 (s, 1H), 8.26 (s, 1H), 7.91–7.82 (m, 3H), 7.67–7.65 (m, 1H), 7.50–7.40 (m, 2H), 7.15 (d, 2H, J = 8.8 Hz), 4.94 (s, 2H); MS(ESI), m/z: [M − MeOH]$^-$.

*5.2.3.23. 2-(4-((4,6-Dioxo-2-thioxotetrahydropyrimidin-5(6H)-ylidene) methyl)phenoxy)-N-phenylacetamide (**4a**).* Yield: 86.3%; HPLC: 99.2%; Yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.40 (s, 1H), 12.31 (s, 1H), 10.18 (s, 1H), 8.41 (d, 2H, J = 7.2 Hz), 8.27 (s, 1H), 7.63 (d, 2H, J = 8.4 Hz), 7.33 (t, 2H, J = 10.0 Hz), 7.14–7.07 (m, 2H), 4.88 (s, 2H); MS (ESI), m/z: 380.03 [M − H]$^-$.

*5.2.3.24. 2-(4-((4,6-Dioxo-2-thioxotetrahydropyrimidin-5(6H)-ylidene)methyl)phenoxy)-N-p-tolylacetamide (**4b**).* Yield: 80.9%; HPLC: 98.2%; Yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.40 (s, 1H), 12.31 (s, 1H), 10.09 (s, 1H), 8.41 (d, 2H, J = 9.2 Hz), 8.27 (s, 1H), 7.51 (d, 2H, J = 8.4 Hz), 7.14–7.12 (m, 4H), 4.86 (s, 2H), 2.26 (s, 3H); MS (ESI), m/z: 426.17 [M − MeOH]$^-$.

*5.2.3.25. 2-(4-((4,6-Dioxo-2-thioxotetrahydropyrimidin-5(6H)-ylidene)methyl)phenoxy)-N-(4-methoxyphenyl)acetamide (**4c**).* Yield: 85.0%; HPLC: 98.7%; Yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.40 (s, 1H), 12.31 (s, 1H), 10.04 (s, 1H), 8.41 (d, 2H, J = 10.2 Hz), 8.27 (s, 1H), 7.55–7.52 (m, 2H), 7.13 (d, 2H, J = 10.2 Hz), 6.92–6.88 (m, 2H), 4.84 (s, 2H), 3.72 (s, 3H); MS (ESI), m/z: 442.10 [M − MeOH]$^-$.

*5.2.3.26. N-(3,4-Dimethoxyphenyl)-2-(4-((4,6-dioxo-2-thioxotetrahydropyrimidin-5(6H)-ylidene)methyl)phenoxy)acetamide (**4d**).* Yield: 77.5%; HPLC: 97.9%; Yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.40 (s, 1H), 12.31 (s, 1H), 10.03 (s, 1H), 8.41 (d, 2H, J = 9.2 Hz), 8.27 (s, 1H), 7.32 (d, 1H, J = 2.4 Hz), 7.17–7.12 (m, 3H), 6.91 (d, 1H, J = 8.8 Hz), 4.84 (s, 2H), 3.73 (s, 3H), 3.72 (s, 3H); MS (ESI), m/z: 440.11 [M − H]$^-$.

*5.2.3.27. 2-(4-((4,6-Dioxo-2-thioxotetrahydropyrimidin-5(6H)-ylidene) methyl)phenoxy)-N-(4-(trifluoromethyl)phenyl)acetamide (**4e**).* Yield: 80.2%; HPLC: 99.3%; Yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.40 (s, 1H), 12.30 (s, 1H), 10.55 (s, 1H), 8.40 (d, 2H, J = 8.8 Hz), 8.26 (s, 1H), 7.85 (d, 2H, J = 8.4 Hz), 7.70 (d, 2H, J = 8.4 Hz), 7.13 (d, 2H, J = 8.4 Hz), 4.93 (s, 2H); MS (ESI), m/z: 480.06 [M − MeOH]$^-$.

*5.2.3.28. N-(4-Acetylphenyl)-2-(4-((4,6-dioxo-2-thioxotetrahydropyrimidin-5(6H)-ylidene)methyl)phenoxy)acetamide (**4f**).* Yield: 99.0%; HPLC: 98.7%; Yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.40 (s, 1H), 12.30 (s, 1H), 10.53 (s, 1H), 8.41 (d, 2H, J = 9.2 Hz), 8.27 (s, 1H), 7.95 (d, 2H, J = 8.8 Hz), 7.77 (d, 2H, J = 8.4 Hz), 7.13 (d, 2H, J = 8.8 Hz), 4.91 (s, 2H), 2.53 (s, 3H); MS (ESI), m/z: 454.32 [M − MeOH]$^-$.

*5.2.3.29. 2-(4-((4,6-Dioxo-2-thioxotetrahydropyrimidin-5(6H)-ylidene) methyl)phenoxy)-N-(3-fluorophenyl)acetamide (**4g**).* Yield: 86.9%; HPLC: 97.9%; Yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.40 (s, 1H), 12.30 (s, 1H), 10.39 (s, 1H), 8.40 (d, 2H, J = 8.8 Hz), 8.26 (s, 1H), 7.60(d, 1H, J = 10.4 Hz), 7.37–7.35 (m, 2H), 7.12 (d, 2H, J = 8.8 Hz), 6.91–6.90 (m, 1H), 4.89 (s, 2H); MS (ESI), m/z: 430.09 [M − MeOH]$^-$.

*5.2.3.30. 2-(4-((4,6-Dioxo-2-thioxotetrahydropyrimidin-5(6H)-ylidene)methyl)phenoxy)-N-(4-fluorophenyl)acetamide (**4h**).* Yield: 82.6%; HPLC: 99.6%; Yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.40 (s, 1H), 12.31 (s, 1H), 10.24 (s, 1H), 8.41 (d, 2H, J = 9.2 Hz), 8.27 (s, 1H), 7.67–7.63 (m, 2H), 7.20–7.18 (m, 2H), 7.13 (d, 2H, J = 8.8 Hz), 4.87 (s, 2H); MS (ESI), m/z: 430.19 [M − MeOH]$^-$.

*5.2.3.31. N-(3-Chlorophenyl)-2-(4-((4,6-dioxo-2-thioxotetrahydropyrimidin-5(6H)-ylidene)methyl)phenoxy)acetamide (**4i**).* Yield: 84.1%; HPLC: 99.8%; Yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.39 (s, 1H), 12.30 (s, 1H), 10.36 (s, 1H), 8.40 (d, 2H, J = 8.8 Hz), 8.26 (s, 1H), 7.82 (s, 1H), 7.51 (d, 1H, J = 8.4 Hz), 7.26 (t, 1H, J = 8.0 Hz), 7.16–7.11 (m, 3H), 4.86 (s, 2H); MS (ESI), m/z: 446.09 [M − MeOH]$^-$.

*5.2.3.32. N-(4-Chlorophenyl)-2-(4-((4,6-dioxo-2-thioxotetrahydropyrimidin-5(6H)-ylidene)methyl)phenoxy)acetamide (**4j**).* Yield: 45.7%; HPLC: 99.7%; Yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.40 (s, 1H), 12.31 (s, 1H), 10.32 (s, 1H), 8.41 (d, 2H, J = 8.8 Hz), 8.27 (s, 1H), 7.66 (d, 2H, J = 8.8 Hz), 7.39 (d, 2H, J = 8.8 Hz), 7.13 (d, 2H, J = 8.8 Hz), 4.86 (s, 2H); MS (ESI), m/z: 446.09 [M − MeOH]$^-$.

*5.2.3.33. N-(4-Bromophenyl)-2-(4-((4,6-dioxo-2-thioxotetrahydropyrimidin-5(6H)-ylidene)methyl)phenoxy)acetamide (**4k**).* Yield: 98.9%; HPLC: 98.8%; Yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.40 (s, 1H), 12.30 (s, 1H), 10.31 (s, 1H), 8.40 (d, 2H, J = 8.8 Hz), 8.26 (s, 1H), 7.61 (d, 2H, J = 8.8 Hz),7.51 (d, 2H, J = 8.4 Hz), 7.12 (d, 2H, J = 8.8 Hz), 4.86 (s, 2H); MS (ESI), m/z: 458.22 [M − H]$^-$.

*5.2.3.34. N-(2,4-Dichlorophenyl)-2-(4-((4,6-dioxo-2-thioxotetrahydropyrimidin-5(6H)-ylidene)methyl)phenoxy)acetamide (**4l**).* Yield: 91.3%; HPLC: 99.6%; Yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.41 (s, 1H), 12.32 (s, 1H), 9.86 (s, 1H), 8.42 (d, 2H, J = 9.2 Hz), 8.28 (s, 1H), 7.81 (d, 1H, J = 8.8 Hz), 7.71 (d, 1H, J = 2.6 Hz), 7.47–7.44 (m, 1H), 7.15 (d, 2H, J = 9.2 Hz), 4.94 (s, 2H); MS (ESI), m/z: 480.16 [M − MeOH]$^-$.

*5.2.3.35. N-(3,5-Dichlorophenyl)-2-(4-((4,6-dioxo-2-thioxotetrahydropyrimidin-5(6H)-ylidene)methyl)phenoxy)acetamide (**4m**).* Yield: 99.1%; HPLC: 97.6%; Yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.41 (s, 1H), 12.31 (s, 1H), 10.50 (s, 1H), 8.41 (d, 2H, J = 8.8 Hz), 8.27 (s, 1H), 7.73 (d, 2H, J = 2.0 Hz), 7.33 (t, 1H, J = 2.0 Hz), 7.14 (d, 2H, J = 9.2 Hz), 4.91 (s, 2H); MS (ESI), m/z: 480.12 [M − MeOH]$^-$.

*5.2.3.36. N-(3-Chloro-4-fluorophenyl)-2-(4-((4, 6-dioxo-2-thioxotetrahydropyrimidin-5(6H)-ylidene)methyl)phenoxy)acetamide (**4n**).* Yield: 86.4%; HPLC: 98.7%; Yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.40 (s, 1H), 12.31 (s, 1H), 10.38 (s, 1H), 8.40 (d, 2H, J = 8.8 Hz), 8.27 (s, 1H), 7.95–7.93 (m, 1H), 7.57–7.54 (m, 1H), 7.40 (t, 1H, J = 8.8 Hz), 7.13 (d, 2H, J = 8.8 Hz), 4.89 (s, 2H); MS (ESI), m/z: 464.20 [M − MeOH]$^-$.

*5.2.3.37. N-(3,4-Difluorophenyl)-2-(4-((4,6-dioxo-2-thioxotetrahydropyrimidin-5(6H)-ylidene)methyl)phenoxy)acetamide (**4o**).* Yield: 72.4%; HPLC: 98.9%; Yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.40 (s, 1H), 12.30 (s, 1H), 10.40 (s, 1H), 8.40 (d, 2H, J = 8.8 Hz), 8.26 (s, 1H), 7.82–7.77 (m, 1H), 7.47–7.38 (m, 2H), 7.12 (d, 2H, J = 8.8 Hz), 4.88 (s, 2H); MS (ESI), m/z: 448.24 [M − MeOH]$^-$.

*5.2.3.38. 2-(4-((4,6-Dioxo-2-thioxotetrahydropyrimidin-5(6H)-ylidene)methyl)phenoxy)-N-methyl-N-phenylacetamide (**4p**).* Yield: 79.4%; HPLC: 98.0%; Yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6):

δ 12.38 (s, 1H), 12.29 (s, 1H), 8.35 (d, 2H, J = 8.0 Hz), 8.24 (s, 1H), 7.50–7.41 (m, 5H), 6.91 (s, 2H), 4.60 (s, 2H), 3.20 (s, 3H); MS (ESI), m/z: 426.23 [M − MeOH]$^-$.

*5.2.3.39. N-Cyclohexyl-2-(4-((4,6-dioxo-2-thioxotetrahydropyrimidin-5(6H)-ylidene)methyl)phenoxy)acetamide (**4q**).* Yield: 87.8%; HPLC: 99.2%; Yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.40 (s, 1H), 12.30 (s, 1H), 8.40 (d, 1H, J = 9.2 Hz), 8.26 (s, 1H), 7.99 (d, 1H, J = 8.0 Hz), 7.06 (d, 2H, J = 9.2 Hz), 4.62 (s, 2H), 3.62–3.60 (m, 1H), 1.74–1.64 (m, 4H), 1.58–1.55 (m, 1H), 1.31–1.19 (m, 4H), 1.15–1.06 (m, 1H); MS (ESI), m/z: 418.11 [M − MeOH]$^-$.

*5.2.3.40. N-Benzyl-2-(4-((4,6-dioxo-2-thioxotetrahydropyrimidin-5(6H)-ylidene) methyl)phenoxy)acetamide (**4r**).* Yield: 77.4%; HPLC: 99.3%; Yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.40 (s, 1H), 12.30 (s, 1H), 8.73 (t, 1H, J = 5.6 Hz), 8.40 (d, 2H, J = 8.4 Hz), 8.26 (s, 1H), 7.33–7.21 (m, 5H), 7.09 (d, 2H, J = 8.4 Hz), 4.73 (s, 2H), 4.35 (d, 2H, J = 5.6 Hz); MS (ESI), m/z: 426.17 [M − MeOH]$^-$.

*5.2.3.41. 2-(4-((4,6-Dioxo-2-thioxotetrahydropyrimidin-5(6H)-ylidene) methyl)phenoxy)-N-(pyridin-2-yl)acetamide (**4s**).* Yield: 88.9%; HPLC: 98.6%; Yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.40 (s, 1H), 12.31 (s, 1H), 10.73 (s, 1H), 8.40 (d, 2H, J = 9.2 Hz), 8.36–8.34 (m, 1H), 8.27 (s, 1H), 8.03 (d, 1H, J = 8.4 Hz), 7.85–7.81 (m, 1H), 7.180–7.15 (m, 1H), 7.11 (d, 2H, J = 9.2 Hz), 4.99 (s, 2H); MS (ESI), m/z: 413.06 [M − MeOH]$^-$.

*5.2.3.42. 2-(4-((4,6-Dioxo-2-thioxotetrahydropyrimidin-5(6H)-ylidene)methyl)phenoxy)-N-(5-methylpyridin-2-yl)acetamide (**4t**).* Yield: 78.2%; HPLC: 99.6%; Yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.40 (s, 1H), 12.30 (s, 1H), 10.57 (s, 1H), 8.40 (d, 1H, J = 8.8 Hz), 8.27 (s, 1H), 8.18 (s, 1H), 7.94 (d, 1H, J = 8.0 Hz), 7.63 (dd, 1H, J = 8.4 Hz), 7.10 (d, 2H, J = 8.8 Hz), 4.95 (s, 2H), 2.26 (s, 3H); ^{13}C NMR (400 MHz, DMSO-d_6): δ 175.19, 169.20, 166.69, 166.45, 157.23, 151.10, 149.41, 137.84, 132.32, 132.01, 129.50, 124.51, 124.19, 118.56, 114.89, 114.29, 114.03, 66.97, 21.23; MS (ESI), m/z: 427.15 [M − MeOH]$^-$.

*5.2.3.43. 2-(4-((4,6-Dioxo-2-thioxotetrahydropyrimidin-5(6H)-ylidene)methyl)phenoxy)-N-(naphthalen-1-yl)acetamide (**4u**).* Yield: 88.6%; HPLC: 99.9%; Yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.40 (s, 1H), 12.31 (s, 1H), 10.24 (s, 1H), 8.44 (d, 2H, J = 8.0 Hz), 8.29 (s, 1H), 8.03–7.94 (m, 2H), 7.81 (d, 1H, J = 8.0 Hz), 7.67–7.65 (m, 1H), 7.56–7.49 (m, 3H), 7.20 (d, 2H, J = 8.4 Hz), 5.05 (s, 2H); MS (ESI), m/z: 462.09 [M − MeOH]$^-$.

*5.2.3.44. 2-(4-((4,6-Dioxo-2-thioxotetrahydropyrimidin-5(6H)-ylidene)methyl)phenoxy)-N-(naphthalen-2-yl)acetamide (**4v**).* Yield: 89.3%; HPLC: 99.3%; Yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.41 (s, 1H), 12.31 (s, 1H), 10.41 (s, 1H), 8.43 (d, 2H, J = 9.2 Hz), 8.32 (s, 1H), 8.28 (s, 1H), 7.91–7.82 (m, 3H), 7.67–7.65 (m, 1H), 7.50–7.40 (m, 2H), 7.16 (d, 2H, J = 8.8 Hz), 4.94 (s, 2H); MS(ESI), m/z: 462.10 [M − MeOH]$^-$.

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Appendix. Supplementary data

Supplementary data related to this article can be found online at doi:10.1016/j.ejmech.2011.02.033.

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