European Journal of Medicinal Chemistry 87 (2014) 595–610

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis and lipid-lowering evaluation of 3-methyl-1*H*-purine-2,6-dione derivatives as potent and orally available anti-obesity agents

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ARTICLE INFO

Article history:
Received 8 August 2014
Received in revised form
25 September 2014
Accepted 29 September 2014
Available online 30 September 2014

Keywords:
Triglycerides
Insulin resistance
Lipid-lowering
Diet induced obesity

ABSTRACT

Obesity accompanied with metabolic disorder is often complicated with a strong link of dyslipidemia and insulin resistance, whose indicator is the excess accumulation of triglycerides (TG) in cells. Consideration the idea of lipid-lowering and improving insulin resistance, 34 novel compounds by combination the xanthine scaffold with the chain of *Rosiglitazone* have been synthesized. Among them, several compounds showed efficiency on reducing TG in 3T3-L1 adipoctyes, and **11c** exhibited the most optimal capacity in lipid-lowering and improving obese clinical symptoms in DIO mice. Furthermore, the hydrochloride of **11c** (**11c**·**HCl**) showed excellent bioavailability, 58.94%, over 2 folds than that (28.03%) of **11c**, and the anti-obesity effect of **11c**·**HCl** at 50 mg/kg dose was better than that of *Metformin* at 150 mg/kg dose in DIO mice, almost reversed HFD to a normal level. Thus, **11c**·**HCl** might be a potent and orally available anti-obesity agent via alleviating the obese clinical symptoms, body fat, improving serum parameters and insulin resistance and TG clearance in liver.

1. Introduction

Obesity can be defined as an excess of body fat. In clinical terms, a surrogate maker for body fat content is the body mass index (BMI), which exceeds 30 kg/m^2 called obesity [1–4]. Obesity and obesity-related disorders, often referred to as metabolic syndrome, have been recognized as major underlying factors of the pathogenesis of such type 2 diabetes (T2D), hypertension, dyslipidemia, atherosclerosis, and certain types of cancer [1–4]. With the dramatically increasing prevalence of obesity around the world [1–4], the American Medical Association classified obesity as a disease in 2013. The main treatment for obesity consists of dieting and physical exercise, but it is very difficult for people to persist in Ref. [5]. Despite a rising worldwide epidemic of obesity, there are currently only two available drugs for the long-term treatment of obesity: *Orilistat* and *Lorcaserin* [6,7], which were limited use because of their austere adverse effects [8,9]. So there is a strong unmet medical need in reliable and efficient drugs targeting anti-obesity properties and hypolipidemic activity.

A major cause of obesity is the accumulation of TG in adipose tissue [10], and increased adipose tissue mass can occur through increase in cell size, cell number, or both [2]. Nowadays, an elevated TG is frequently the most available laboratory marker of intracellular accumulation of lipids [11]. Impaired fasting glucose often is found together with an increase TG level, which can interrupt insulin action by multiple mechanisms and lead to insulin resistance ultimately [12]. Furthermore, an increasing TG in plasma also could active adipocytokines to increase the risk of coronary heart disease (CHD) [13]. Therefore, the ability to reduce intracellular TG concentrations has been proposed as an attractive therapeutic paradigm for obesity and related diseases.

Abbreviations: CHD, coronary heart disease; DMF, dimethylformamide; PE, petroleum ether; H&E, hematoxylin and eosin; DIO, diet-induced obesity; AICAR, acadesine 5′-monophosphate; Met, metformin; RSG, Rosiglitazone; HFD, high fat diet; TG, Triglycerides; FBG, fasting plasma glucose; ALT, alanine transaminase; AST, aspartate amino-transferase; LDL, low-density lipoprotein; HDL, high-density lipoprotein; FFA, free fatty acids; OGTT, oral glucose tolerance test; ITT, insulin tolerance test; IR, insulin resistance; HOMA-IR, homeostasis model of assessment for insulin resistance index; NAFLD, nonalcoholic fatty liver disease; DIAD, Diisopropyl azodicarboxylate; DMF-DMA, Dimethoxy-N,N-dimethylmethanamine; H&E, hematoxylin-eosin.

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http://dx.doi.org/10.1016/j.ejmech.2014.09.094
0223-5234/

It has been established that insulin resistance is a major factor linking obesity, particularly central adiposity [12]. The insulin signaling system plays an important role in regulating some physiological processes of obesity, including glucose uptake, TG synthesis, lipolysis and adipocytokine secretion [14]. Conversely, excess lipid accumulation is a major cause of insulin resistance in obesity [15]. Prolonged insulin resistance may eventually result in β cell failure and circulating insulin levels become insufficient to control blood glucose, leading to overt hyperglycaemia, hyperlipidemia and relative complications [16]. That is to say, potent and novel insulin regulators have been regarded as therapeutic potential for the treatment of obesity [17].

Xanthine is a purine base found in most human body tissues and fluids and in other organisms [18]. Lots of drugs contain xanthine, such as *Theobromine* [19], *Caffeine* [20] and *Linagliptin* [21], which could improve the blood lipid profile and thereby to help obese persons lose weight, and may account for improved insulin sensitivity. *Rosiglitazone* (RSG), a wonderful insulin sensitizer by binding to the peroxisome proliferators-activated receptor-γ, promoting synthesis of glucose transporters and activating adipocyte differentiation [22]. Based on the structure–activity relationship (SAR) data reported in the literature it appears that the acidic head group may be one of the factors responsible for side effects of RSG [23]. Researches also confirmed that replacement the acidic thiazolidinedione head group of RSG with other heterocycles could obtain significant bioactivities like anti-hyperglycemic and anti-dyslipidemic [23,24]. Encouraged by these results above and based on the idea of lipid lowering agents with improving insulin resistance, we have designed and synthesized a series of novel compounds where the acidic thiazolidinedione head group of RSG was replaced with xanthine scaffold as shown in Fig. 1. 34 novel derivatives were synthesized and evaluated for the ability on reducing TG level in 3T3-L1 adipocytes as well as alleviating obese clinical symptoms in DIO mice.

2. Result and discussion

2.1. Chemistry

The preparation of a library of 3-methyl-8-(4-(2-(methyl(pyridin-2-yl)amino) ethoxy)phenyl)-1*H*-purine-2,6(3*H*,7*H*)-dione derivatives (**11**) have been carried out in a tandem nine-step sequence from two important intermediates **3** and **9** as described in Scheme 1. Compound **2** was afforded by treatment 2-chloropyridine (**1**) and 2-(methylamino)ethanol in a good yield. The pivotal aldehyde intermediate **3** was synthesized via a reaction of **2** and 4-fluorobenzaldehyde in the presence of *t*-BuOK as a base in DMF. The other diketone intermediate (**9**) was synthesized from the most primitive material 1-methylurea (**4**) Treatment of **4** and 2-cyanoacetic acid at 60 °C in DMF, afforded white solid in a good yield, then 20% NaOH solution was added to dissolve the white solid, refluxing for another 2 h, taken the solution in icy water and acidified to pH < 7 to give **5**. Primary amine **5** was protected by DMF-DMA to provide **6** with reaction selectivity. The **6** was treated with NaH, LiBr and various halides in ice-bath to give **7**. Subsequently, **8** was synthesized through two methods of deprotection employing concentrated HCl or aqueous ammonia in ethanol, respectively. The diamino uracil **9** was obtained by its conversion to nitroso racil and reduction with sodium dithionite. Condensation of **3** with **9** in EtOH/AcOH (4/1, v/v) afforded the corresponding amino derivatives, which, on subsequent ring closure in refluxing toluene in the presence of diisopropyl azodicarboxylate (DIAD), provided the corresponding xanthine derivatives **10**. Finally, target compounds **11a**–**x** were obtained by alkylation with various kinds of halide and K_2CO_3, summarized in Table 1.

To explore the SAR, these novel *N*-1 or *N*-7 mono-substitution and *N*-1,7-disubstituted derivatives were synthesized as outlined in Scheme 2. Compound **13** was prepared according to the same synthetic procedures reported for **10**. For the synthesis of **14a**–**d**, controlling the equivalent ratio of halides was critical, one equivalent to **14a**–**b** while two equivalents to **14c**–**d**. The position *N*-7 of **13** was protected by POM-Cl in the presence of K_2CO_3 as a base in anhydrous DMF, then used halides to attack *N*-1 in the same way to make **15a**–**c**. Finally, deprotection of the POM group and degreasing reaction with **15b**–**c** were efficiently achieved by using 2 M HCl in MeOH, thus to gain carboxylic acid derivatives **16a**–**b**. At this stage, all the final products were fully analyzed and characterized by nuclear magnetic resonance (NMR), mass spectrum (MS), and high performance liquid chromatography (HPLC) before entering the biological test.

2.2. Biological evaluations

2.2.1. Determination the content of TG in 3T3-L1 adipocytes

TG is commonly used as indicators of intracellular accumulation of lipids [25,26]. 3T3-L1 mouse preadipocytes are known to differentiate into mature adipocyte-like cells *in vitro*, and intracellular accumulation of lipid droplets has been observed in cell differentiation [27]. All these compounds were evaluated for the inhibitory effects on TG accumulation of 3T3-L1 adipocytes. Acadesine 5′-monophosphate (AICAR), Met, well known inhibitors to reduce the TG level, were selected as positive controls [12]. On the

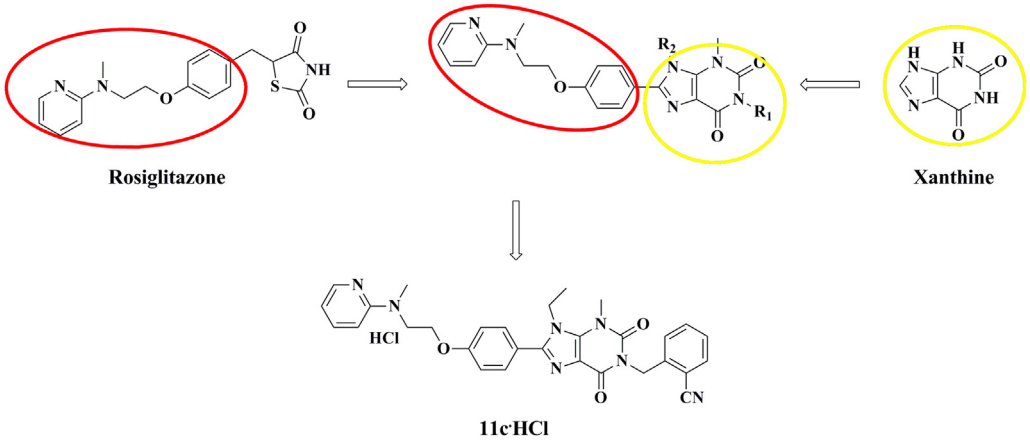
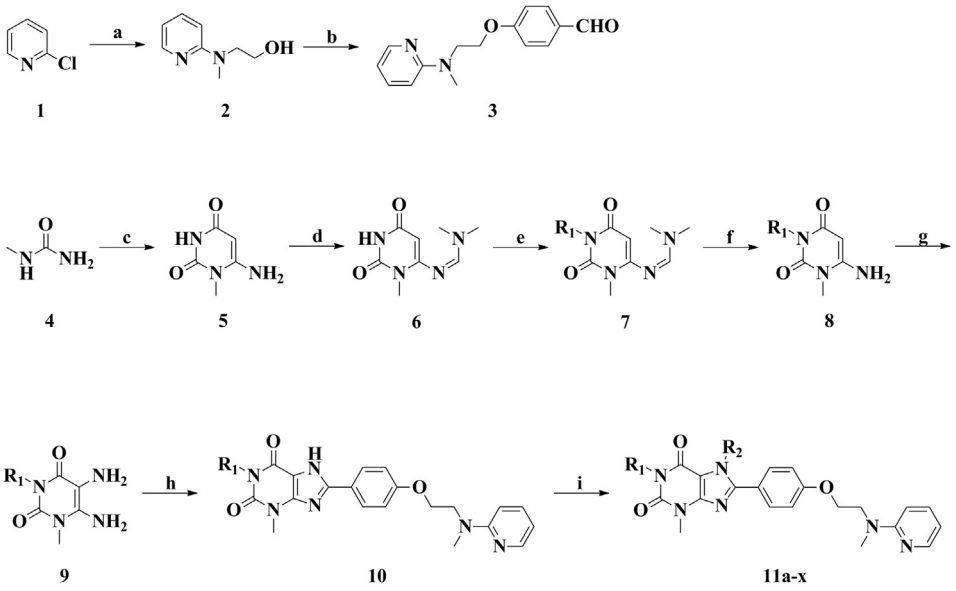


Fig. 1. Design compounds with anti-obesity activity.



Scheme 1. Reagents and conditions: (a) 2-(methylamino)ethanol, 150 °C, 8 h; (b) *t*-BuOK, DMF, 4-fluorobenzaldehyde, 80 °C, overnight; (c) 2-cyanoacetic acid, Ac_2O, 60 °C, 5 h, then 20% NaOH (aq.), 2 h; (d) DMF-DMA, DMF, 40 °C, overnight; (e) R_1-X, LiBr, NaH, DMF, 70 °C, overnight; (f) concentrated HCl or 28% NH_4OH, EtOH, 40 °C, 5 h; (g) AcOH, $NaNO_2$, 0 °C to room temperature, 5 h; then MeOH, NH_4OH, $Na_2S_2O_4$, 60 °C, 3 h; (h) **3**, EtOH, AcOH, reflux, then DIAD, toluene, reflux, 5 h; (i) R_2-X, K_2CO_3, DMF, 70 °C, over 5 h.

contrary, RSG, a lipogenic enhancer, was chosen as negative control [27].

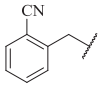
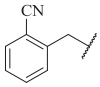
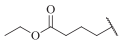
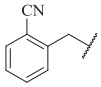
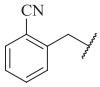
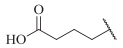
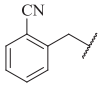
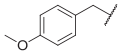
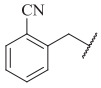
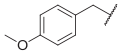
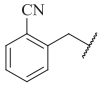
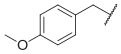
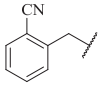
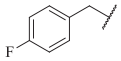
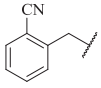
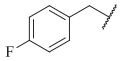
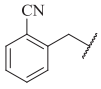
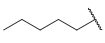
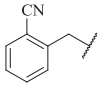
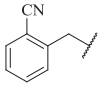
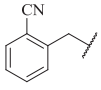
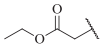
As depicted in Fig. 2, **11c**, **11d**, **11f**, **11j**, **14d** and **15a** significantly suppressed the accumulation of TG in mature 3T3-L1 cells at 10 μM. Among them, **11c** and **d** were considered as identified hits with a favorable TG inhibition ratio 64.47% and 51.11%, which were higher than AICAR 50.96%. **11a**, without any substituent group at *N*-7, showed no biological activity. However, the introduction of an alkyl substituent led to an increase in lowering TG, and decreased roughly when the bulk of substituent group increased by comparison with **11b**–**n**. The order of potency was 1-ethyl > 1-propyl > 1-methyl > others, which was related to their poor solubility as well as high hydrophobicity. In order to increase water solubility of the highly lipophilic, polar groups, such as acidic or basic functions, were introduced into *N*-7 position. As for **11k**–**n**, the exposed carboxylic group revealed a better potency in restraining adipogenesis than the corresponding ethyl ester compounds. Similar cases were observed with **14c** and **d**, but all of them did not show potency improvements over **11c** or **d**. Further elaboration to small alkanes at *N*-1 position (**11o**–**x**) did not improve potency, neither. Nevertheless, this unfavorable result also confirmed the order of potency was 1-ethyl > 1-propyl by contrasting **11p**–**s** or **11w**–**x**. Quite different from the aryl group at *N*-1 position, the introduction of alkyl substituent at *N*-7 resulted in decreasing activity while *N*-1 was small alkanes. By contrast, the presence of the cyano group on the terminal phenyl of **11c** and **d** led to reduce the hydrophobicity and enhance of inhibitor potency. Also, there was not any appreciable hit among the derivatives without substituent group at *N*-1, except **15a**, which was particularly beneficial for potency by the ester segment that reduced hydrophobicity. In spite of **15b**–**16b** did not exhibit a good efficacy in lowering TG, the data also confirmed that *N*-1,7-disubstituted was a necessary portion for this series compounds. Besides, reducing the high lipophilicity was even important from the *N*-1,7-disubstituted compounds **11u**, **11x**, **14c** and **d**. Thus, the structure activity relationship of 3-methyl-1*H*-purine-2,6-dione Derivatives in suppression the accumulation of TG in mature 3T3-L1 cells could simply summary here: 1) when *N*-1 was aryl group, the order of potency was 1-ethyl > 1-propyl at *N*-7 position; 2) when *N*-1 was small alkanes, the efficacy of no substitutes at *N*-7 position was better than there was substitutes; 3) when *N*-1 was no substitute group, the introduction of polar group at *N*-7 was necessary.

2.2.2. *Effects of **11c** and **d** in diet-induced obesity (DIO)*

On the basis of their excellent potency of lowing TG *in vitro*, **11c** and **d** were selected for further pharmacologic evaluation in DIO model with C57Bl/6J mice [11,28]. Oral administration of high-fat diet (HFD, Research Diets, D12492) for 12 weeks, the mice were successfully induced obesity as evidenced by the gain of body weight compared to the normal group. At the same time, the male rats were in single-dose acute toxicity assessment, the maximal feasible single oral dose, 2.5 g/kg, showed no significant effect on clinical signs, or body weight and mortality were observed over a 7-day period in sub-acute toxicity measurement (Supporting information). According to the results of *in vitro* assay and previous experimental dosage, we accepted a dose of 50 mg/kg/day as a therapeutic strategy. After oral administration of HFD + **11c** (50 mg/kg/day), HFD + **11d** (50 mg/kg/day), and HFD + Met (150 mg/kg/day) for another 4 weeks, the average body weight was reduced respectively by 15.96%, 7.64% and 19.08% without dramatically changes of food intake compared to the HFD group. The mean loss of liver weight was 16.80%, 12.80% and 23.20%, while the mean fat mass loss of epididymal fat tissues was 21.65%, 4.76% and 22.94%, respectively. As a comparison, **11c** exhibited superior ability in controlling the weight of body, liver and fat than **11d** from all the results above, which was close to Met (Fig. 3).

Obesity is not only a major contributor to the metabolic dysfunction involving lipid, but also blood glucose and insulin [29]. In order to investigate the potential effects of two candidate compounds on blood glucose and insulin homeostasis, an oral glucose tolerance test (OGTT) and insulin tolerance test (ITT) were carried out on DIO mice after 4-week efficacy study. Such two disease models develop obese and obesity-related features such as impaired glucose tolerance, hyperinsulinemia and hyperlipidemia [30]. **11c** showed a significant improvement in glucose tolerance and insulin resistance at a 50 mg/kg dose ($P < 0.05$), especially in abdominal case (Fig. 4B, **4D**), which was similar to Met, and **11d** was negative. The time of reducing plasma glucose concentration to the

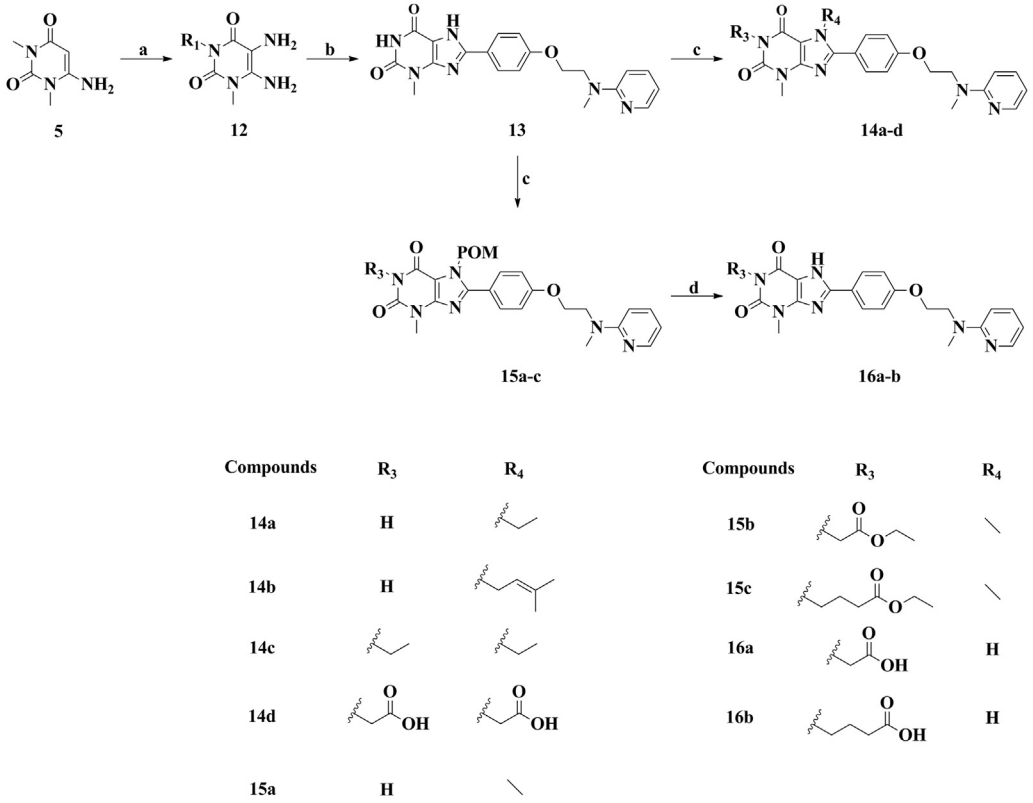
Table 1
Structural information of **11** for pharmacological profilings.

No.	R_1	R_2	No.	R_1	R_2
11a		**H**	**11m**		
11b			**11n**		
11c			**11o**		**H**
11d			**11p**		
11e			**11q**		
11f			**11r**		
11g			**11s**		
11h			**11t**		**H**
11i			**11u**		
11j			**11v**		**H**
11k			**11w**		
11l			**11z**		

initial status by 50% exceed 40 min and the rate of decline in plasma glucose concentrations every min (KTT) was >2%/min, so there was little risk of hypoglycemia. Accordance with the OGTT test that **11c** did not alter fasting glucose level with normal glucose homeostasis. So, **11c** that eliminated excess lipid accumulation (in liver and muscle) could improve glucose homeostasis by reversing lipid-induced insulin resistance and alleviate obesity [12].

At the end of the feeding study, fasting plasma blood (FBG) and insulin levels were tested, and the homeostasis model assessment of basal insulin resistance (HOMA-IR) was calculated. Lower HOMA-IR values indicate greater insulin sensitivity, whereas higher HOMA-IR values show lower insulin sensitivity or insulin resistance [11]. As shown in Table 2, **11c** significantly reversed HFD diet induced elevations in plasma glucose (39.48%) and insulin (42.02%) levels compared to obese controls. HOMA-IR was found to be higher in HFD and HFD + **11d** groups. However, insulin sensitivity was increased by **11c** supplementation as indicated by lower HOMA-IR. These results were consistent with the ITT study that showed above.

Subsequently, we also measured other biochemical parameters in serum level, including alanine transaminase (ALT), aspartate aminotransferase (AST), TG, low-density lipoprotein (LDL), high-density lipoprotein (HDL) and free fatty acids (FFA), which were primary associated with dyslipidemia and obesity.[7] HFD mice showed an increasing level of plasma AST, ALT, TG, LDL, HDL and FFA, which were gradually reduced in **11c** mice by 83.67%, 10.28%, 6.42%, 5.88%, 106.32% and 3.82%, respectively. The decrease TG content may result from decreasing lipid synthesis and further increased in insulin sensitivity in **11c** mice. **11d** did not show noticeable effect in these changes. Moreover, the ALT and AST levels of **11c** treated mice were similar to that of normal and Met treated groups. To put it simply, **11c** could improve plasma lipid profile and scarcely cause any hepatotoxicity, which was correlated with lower adipose tissue and liver mass.



Scheme 2. Reagents and conditions: (a) AcOH, $NaNO_2$, 0 °C to room temperature, 5 h, then MeOH, NH_4OH, $Na_2S_2O_4$, 60 °C, 3 h; (b) **3**, EtOH, AcOH, reflux, 4 h, then DIAD, toluene, reflux, 5 h; (c) RX, K_2CO_3, anhydrous DMF, 70 °C, over 5 h; (d) 2 M NaOH, MeOH, 80 °C, overnight.

Up till now, Oil Red O staining has been commonly used to screen the changes in lipid content. However, there is concern about the specificity and the inaccuracy of the results obtained from the Oil Red O staining [31]. Therefore, the liver sections were not only processed routinely for Oil Red O assay, but also together with hematoxylin staining. Besides, the tissues of liver and fat also stained with hematoxylin-eosin (H&E) thus to make the result reliable. In agreement with a decline in the content of hepatic TG, there were numerous lipid droplets in liver tissues from HFD-induced mice severely ruined liver histology presented as ballooning degeneration and further led to the formation of steatosis. Fortunately, the reduction of hepatic TG by the administration of **11c**, **d** and Met was 7.17%, 5.15% and 10.73%, respectively. Similar results were seen in Fig. 5B–D, the fat accumulation from liver tissues of treated mice (HFD + Met, HFD + **11c**) was distinctly alleviated, and the remarkable reduction of the number and size of lipid droplets was observed (black arrowheads). Consistently with the results above, **11c** displayed better capacity of controlling the differentiation and size of adipocytes than **11d**, which had minimal efficacy.

2.2.3. *Pharmacokinetics of **11c** and **11c**·HCl*

With the preferable *in vivo* evaluation, **11c** was investigated the pharmacokinetics in Sprague–Dawley rats, whose bioavailability was only 28.03% (Table 3). However, the poor oral bioavailability of **11c** may have a close relationship with such a high oral dosage in DIO model. Thus we tried to prepare various salts of **11c**, such as

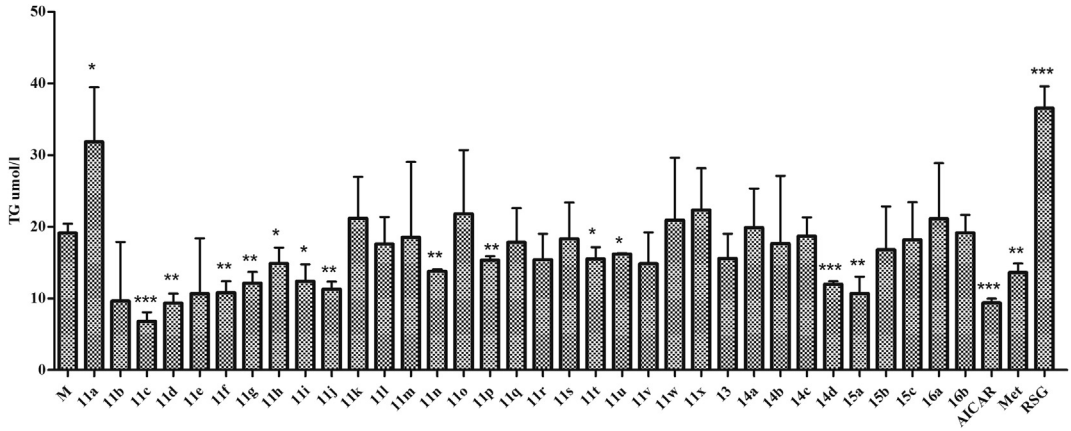


Fig. 2. Inhibitory effects of 34 novel compounds on TG accumulation in 3T3-L1 adipocytes. RSG and compounds were at a same concentration of 10 μM, whereas Met was 1.0 mM and AICAR was 0.5 mM. Results were recorded from three independence experiments: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ *vs.* the corresponding control.

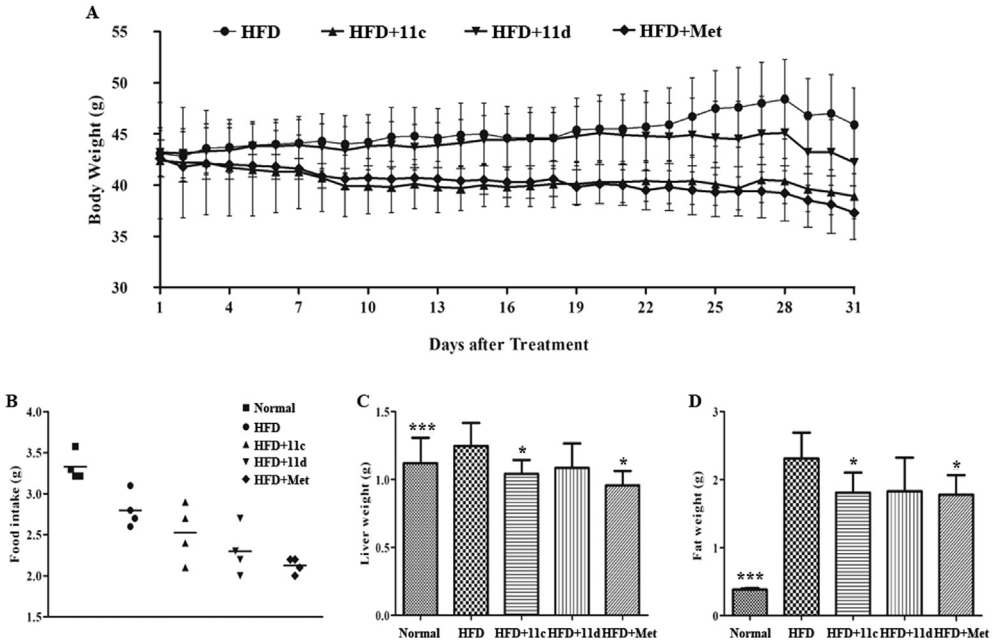


Fig. 3. **11c** and **d** improved the metabolic symptoms in DIO mice. (A) Body weights of DIO mice, either HFD group ($n = 6$) or treated groups ($n = 6$), were monitored the oral administration of **11c** (50 mg/kg/day), **11d** (50 mg/kg/day) and Met (150 mg/kg/day) for 4 weeks. (B) food intake, (C) liver weight, and (D) fat weight (epididymal and abdominal fat mass) were analyzed: #, $P < 0.05$ *vs.* normal group; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ *vs.* the corresponding HFD group.

trifluoroacetic acid salt, benzoate, maleate and hydrochloride. Only hydrochloride was prepared successfully owing to the weak alkaline of **11c**. As expected, **11c**·**HCl** had an amazing solubility of 3.28 mg/mL at room temperature, whereas **11c** was only 1 mg/mL (Supporting information). Agree with the result of solubility, the bioavailability of **11c**·**HCl** was also ascended to 58.94%, over 2-fold of **11c**. Despite the intravenous half-life of hydrochloride was shorter than primary compound, the oral half-life was a slight longer, approximately 7 h. The delta-T between **11c** and **11c**·**HCl** was only 0.6 h, which implied **11c**·**HCl** should be hydrolyzed to **11c** quickly and taken effect. Besides, acute and sub-acute toxicity studies of **11c**·**HCl** in Sprague–Dawley rats in series doses up to

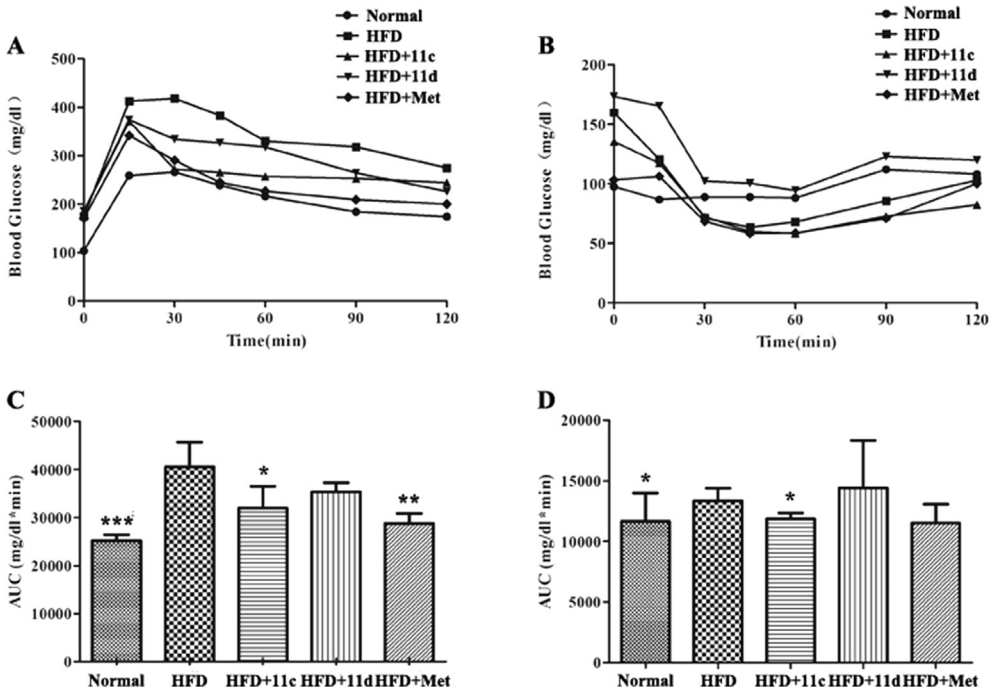


Fig. 4. Effects of **11c** and **d** on postprandial glucose levels in OGTT and ITT. Both of these tests were performed in treated DIO mice for 4 weeks after one night of fasting. Blood was sampled from the tail vein of mice at 0, 5, 15, 30, 60, 90 and 120 min after an oral glucose load of 2.0 g/kg or intraperitoneally insulin load of 0.5 U/kg of body weight. Top panels show plasma glucose concentration curve as a function of time and bottom panels show areas under the curves: (A, C) blood glucose level in OGTT and (B, D) blood glucose level in ITT. Results were the mean ± standard errors ($n = 6$) as shown: #, $P < 0.05$ *vs.* normal group; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ *vs.* the corresponding HFD group.

Table 2
Parameters of biochemical markers from DIO mice.[a]

Parameter	Normal	HFD	HFD + **11c**	HFD + **11d**	HFD + **Met**
FBG (mM)[b]	5.0 ± 1.30*	8.51 ± 2.19	5.15 ± 0.63*	7.25 ± 1.74	5.32 ± 1.14*
Insulin (mU)	11.85 ± 2.6	9.40 ± 1.0	13.35 ± 3.1*	12.04 ± 2.5	8.10 ± 1.2
ALT (U/L)	33.00 ± 18.23**	122.50 ± 38.89	20.00 ± 8.66**	86.00 ± 55.39	21.67 ± 2.89*
AST (U/L)	125.00 ± 35.00	133.75 ± 27.20	120.00 ± 28.72	150.00 ± 23.18	110.00 ± 30.00
TG (Mm)	0.93 ± 009*	1.09 ± 0.11	1.02 ± 0.16	1.15 ± 0.06	0.92 ± 0.14
LDL (Mm)	0.14 ± 0.07	0.17 ± 0.06	0.11 ± 0.01*	0.16 ± 0.03	0.12 ± 0.03
HDL (Mm)	0.60 ± 0.17**	0.95 ± 0.04	1.96 ± 0.07*	0.96 ± 0.15	0.82 ± 0.09**
FFA (ng/L)	19.55 ± 1.57	23.57 ± 2.44	22.67 ± 3.05	24.43 ± 5.61	21.56 ± 1.26
HOMA-IR	2.61 ± 0.85	3.50 ± 0.71	3.06 ± 0.85	3.99 ± 1.65	1.93 ± 0.54**

HOMA-IR = fasting plasma glucose (mmol/L) × fasting insulin (mU)/22.5.
[a] The HFD-fed group ($n = 6$) or treated groups ($n = 6$) were monitored during the oral administration of **11c** (50 mg/kg/day), **11d** (50 mg/kg/day) and Met (150 mg/kg/day) for 4 weeks. The results as were expressed as the mean ± S.D.
[b] Fasting blood glucose:*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ *vs*. the corresponding HFD group.

2.5 g/kg exhibited no toxicity according to measurements of both physiological and metabolic variables. Based on these advantages of **11c·HCl**, it was also selected for further evaluation in DIO model.

2.2.4. *Effects of **11c·HCl** in DIO mice*

To investigate the further effect of **11c·HCl** on adipocytes content *in vivo*, we employed the DIO model where we administered two doses of **11c·HCl** (20 and 50 mg/kg/day) for 4 weeks. During or after the treatment period, all the phenomenon and data revealed that the curative effect of high-dose **11c·HCl** was better than that of Met treatment group in contrast to ones of HFD ($P < 0.05$) without affecting dramatic changes in accumulative food intake (Fig. 6A, B). Among them, the average body weight was reduced respectively by 12.86%, 15.08% and 11.41%, the mean mass of liver decreased respectively by 20.00%, 22.67% and 22.00% (Fig. 6C), along with the fat loss was by 18.18%, 56.20% and 9.92% respectively (Fig. 6D). Particularly, the result of fat loss in high-dose **11c·HCl** group came close to the normal group. That was to say, the high-dose of **11c·HCl** owned the best capacity in improving obesity and no abnormal behavioral or physiological signs were observed during the experiment.

A combination of excellent *in vivo* activity, dramatically improved pharmacokinetic profile, and minimal risk of cytotoxicity profiles, **11c·HCl** was assessed for its ability to improved glucose tolerance and insulin resistance in DIO mice after 4-week treatment. As expected, single oral doses of **11c·HCl** robustly lowered the blood glucose (Fig. 7A, C) and augmented insulin secretion (Fig. 7B, D) during the tests in a dose-proportional manner from 20 to 50 mg/kg. It should be noted that **11c·HCl** exhibited a significant blood glucose-lowering effect at two doses in comparison with Met. Even at the high dose, **11c·HCl** did not alter fasting glucose levels in DIO mice with normal glucose homeostasis, which was in accordance with the absence of hypoglycemic effects. Moreover, **11c·HCl** displayed a much stronger capacity in glucose-lowering after the administration of insulin at all times points than Met, a well-known biguanide drug that increases insulin sensitivity [24].

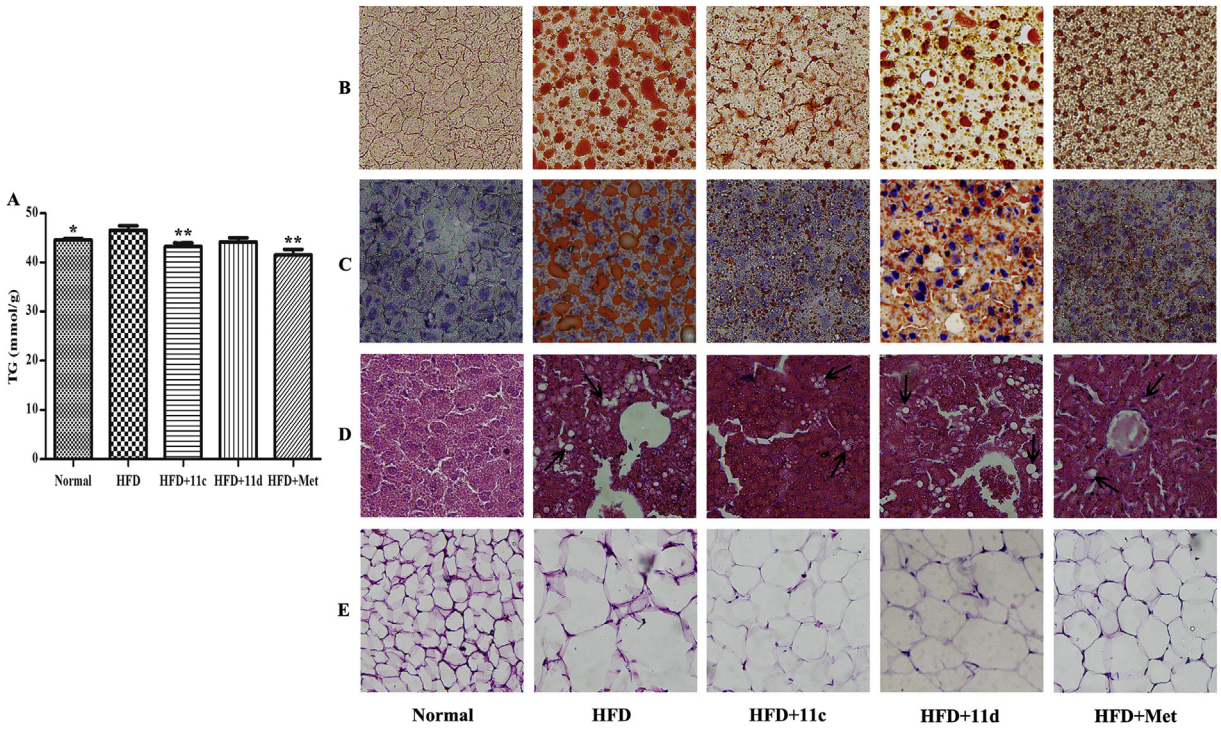


Fig. 5. (A) Effects of **11c** and **d** on hepatic TG content from DIO mice. The values represent the mean ± S.D from 6 mice in each group: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ *vs*. the corresponding HFD group. Histological examination of liver and fat (epididymal visceral adipose tissue) samples from DIO mice: (B) Oil Red O staining and (C) Oil Red O + hematoxylin staining of representative liver sections, along with H&E staining of (D) liver and (E) fat tissues (scale, 200×). Lipid droplets were marks using black arrowheads.

Table 3
Pharmacokinetic profiles of **11c** and **11c·HCl** in Mice. Data were mean concentrations in mouse plasma ($n = 5$) following a single 5.0 mg/kg intravenous dose or 20 mg/kg oral dose.

		11c	**11c·HCl**
Intravenous	C_{max} (μg/L)	341.544	1741.815
	t_{max} (h)	0.083	0.083
	$t_{1/2}$ (h)	8.829	5.817
	AUC_{0-t} (μg/L·h)	404.149	1778.411
	V_d (L/kg)	142.052	23.626
	CL_{total} (L/h/kg)	11.314	2.825
Oral	C_{max} (μg/L)	54.412	465.159
	t_{max} (h)	2.267	2.792
	$t_{1/2}$ (h)	6.304	6.973
	AUC_{0-t} (μg/L·h)	453.074	4192.938
	V_d (L/kg)	416.501	67.208
	CL_{total} (L/h/kg)	61.512	8.31
F (%)		28.03	58.94

In short, **11c·HCl** could not only lower the concentration of plasma glucose, but also improve insulin resistance. These encouraging results in terms of reduced glucose excursion in addition to the effect on insulin levels and on lowering body weight gain suggested that **11c·HCl** could provide potential benefits for treating obesity and diabetes.

Similar observation was found by comparison some important biochemical makers in plasma level after the treatment. In contract to HFD group, the treatment of **11c·HCl** effectively recovered serum biomarkers to appropriate ranges and decreased HOMA-IR dramatically in a dose dependent manner. Specially, high-dose **11c·HCl** presented ascendant improvements of TG (28.34%), LDL (45.16%), HDL (112.31%), FFA (20.46%) and HOMA-IR (59.34%), better than that in Met group, almost reversed HFD diet to a normal level (Table 4).

The content of TG in liver, morphology of adipose tissue and liver of mice from different diet groups is depicted in Fig. 8. Compared to HFD mice, the reduction of hepatic TG by the administration of **11c·HCl** and Met was 16.80% (20 mg/kg/day), 21.59% (50 mg/kg/day) and 20.98%, respectively. Remarkably, the cytoplasm of the hepatocytes from HFD mice showed microvesicular vacuolation (the presence of numerous lipid droplets), as well as hypertrophic, clear adipocytes of fat tissues, suggesting that the diet feeding led to an excessive fat deposition and accumulation. However, **11c·HCl** supplementation altered adipose tissue morphology and reduced lipid accumulation in the liver, according with a decline in the content of TG in liver. Especially for the high dose, **11c·HCl** revealed prominent efficacy in improving the extent of fatty change, which displayed normal morphology nearly without obvious histology changes and site of steatosis. Low-dose **11c·HCl** fed mice had lower lipids in hepatic tissues and decreased size of fat cells as similar seen in ones from Met group. These data are parallel with the restricting body and liver tissue weights as a consequence of reduction in fat and TG content of DIO mice showed above. Obviously, **11c·HCl** possessed the potential for weight lowing effect on obese symptoms and improved the obesity related metabolic features.

3. Conclusion

In this study, we have developed a series of 3-methyl-1*H*-purine-2,6-dione derivatives as novel lowering TG agents. Our structure–activity relationship study was clear and revealed that compounds with appropriate polarity exhibited good efficacy on lowering the synthesis of TG in 3T3-L1 adipocytes. Of these molecules, compound **11c** showed TG reduction and improved the obese clinical symptoms when studied *in vivo* at a dose of 50 mg/kg/day, which was found to be almost equi-efficacious to Met. Further improvement on solubility and bioavailability resulted in the identification of **11c**-hydrochloride (**11c·HCl**). Thus treatment with **11c·HCl** at two doses of 20 and 50 mg/kg/day in DIO mice revealed good anti-obesity effect. Particularly at high dose, **11c·HCl** markedly alleviated the clinical symptoms of obesity, the changes of TG, LDL, HDL, FFA and fat mass were reduced to normal group, and histological evaluation displayed normal condition. Besides, there were no significant adverse effects were observed in toxicity study with rats. Overall, potent and safe lipid-lowering compound, **11c·HCl** can be expected to provide a novel therapeutics for obesity-related disease without any risk of hypoglycemia and toxicity.

4. Experimental section

4.1. General synthetic methods

Chemistry reagents of analytical grade were purchased from Changzheng Chemical Factory, Chengdu, Sichuan, P. R. China. TLC was performed on 0.20 mm Silica Gel 60 F_{254} plates (Qingdao Ocean Chemical Factory, Shandong, China). Hydrogen nuclear magnetic resonance (^{1}H NMR) spectra were recorded at 400 MHz while carbon nuclear magnetic resonance (^{13}C NMR) spectra were recorded at 100 MHz on a Varian spectrometer (Varian, Palo Alto, CA) 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; brs, broad 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

Table 4
Parameters of biochemical markers from DIO Mice.[a]

Parameter	Normal	HFD	HFD + **11c·HCl**-20	HFD + **11c·HCl**-50	HFD + **Met**
FBG (mM)	4.88 ± 0.75***	10.09 ± 1.12	8.42 ± 1.91	7.76 ± 1.68*	7.57 ± 2.02*
Insulin (mU)	4.31 ± 0.82***	8.77 ± 2.85	7.48 ± 3.45	4.76 ± 2.39**	5.48 ± 2.60*
ALT (U/L)	29.60 ± 8.29	62.00 ± 10.53	24.00 ± 14.29	18.86 ± 3.80	46.29 ± 4.53
AST (U/L)	128.00 ± 20.59	184.67 ± 14.12	125.71 ± 16.72	131.43 ± 16,72	172.00 ± 9.42
TG[b] (Mm)	0.96 ± 0.14**	1.31 ± 0.11	1.25 ± 0.25	0.94 ± 0.33*	1.07 ± 0.18*
LDL (Mm)	0.13 ± 0.02**	0.31 ± 0.09	0.22 ± 0.03*	0.17 ± 0.03**	0.17 ± 0.06**
HDL (Mm)	1.02 ± 0.13	0.65 ± 0.36	1.31 ± 0.26**	1.38 ± 0.30**	1.38 ± 0.20***
FFA (ng/L)	25.07 ± 3.49**	32.35 ± 7.19	26.80 ± 11.13	25.73 ± 1.42**	26.05 ± 6.70*
HOMA-IR	0.94 ± 0.23***	3.91 ± 1.37	2.98 ± 1.53	1.59 ± 1.03***	1.73 ± 0.91***

HOMA-IR = fasting plasma glucose (mmol/L) × fasting insulin (mU)/22.5.

[a] The HFD-fed group ($n = 6$) or treated groups ($n = 6$) were monitored during the oral administration of **11c·HCl** (20 mg/kg/day), **11c·HCl** (50 mg/kg/day) and Met (150 mg/kg/day) for 4 weeks. The results as were expressed as the mean ± S.D.

[b] Fasting blood glucose: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ *vs*. the corresponding HFD group.

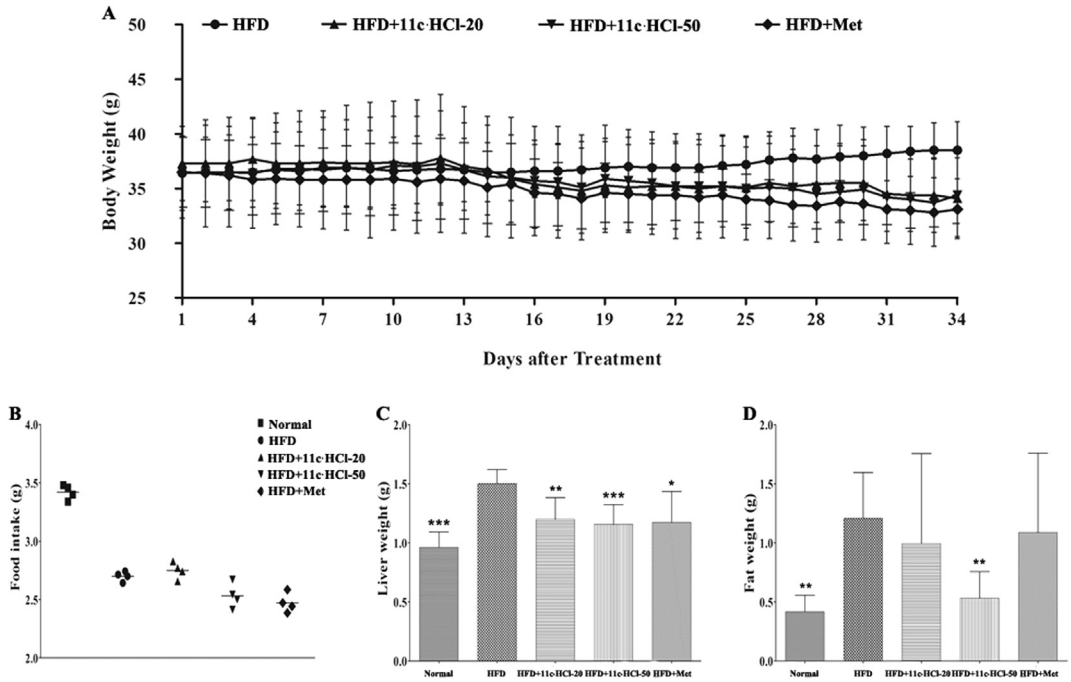


Fig. 6. 11c·HCl improved the metabolic symptoms in DIO mice. (A) Body weights of DIO mice, either HFD group ($n = 6$) or treated groups ($n = 6$), were monitored the oral administration of **11c·HCl** (20 mg/kg/day), **11c·HCl** (50 mg/kg/day) and Met (150 mg/kg/day) for 4 weeks. (B) Food intake, (C) liver weight, and (D) fat weight (epididymal and abdominal fat mass) were analyzed: #, $P < 0.05$ *vs.* normal group; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ *vs.* the corresponding HFD group.

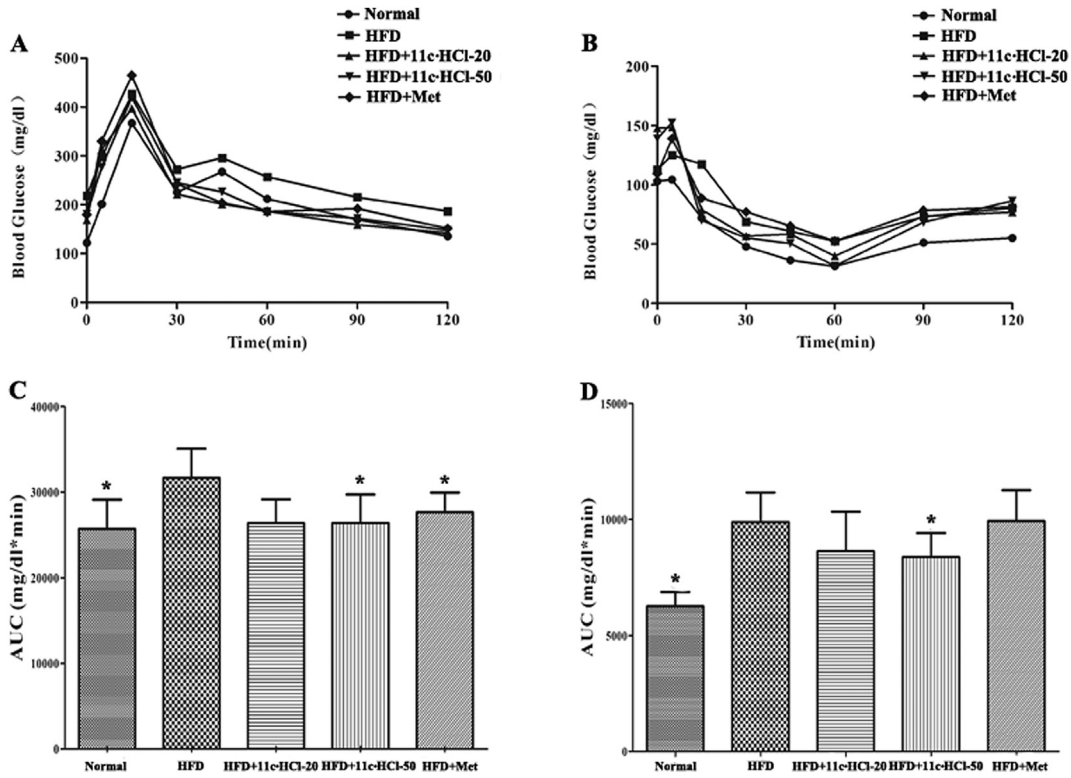


Fig. 7. Effects of **11c·HCl** on postprandial glucose levels in OGTT and ITT. Both of these tests were performed in treated DIO mice for 4 weeks after one night of fasting. Blood was sampled from the tail vein of mice at 0, 5, 15, 30, 60, 90 and 120 min after an oral glucose load of 2.0 g/kg or intraperitoneally insulin load of 0.5 U/kg of body weight. Top panels show plasma glucose concentration curve as a function of time and bottom panels show areas under the curves: (A, C) Blood glucose level in OGTT and (B, D) blood glucose level in ITT. Results were the means ± standard errors ($n = 6$) as shown: #, $P < 0.05$ *vs.* normal group; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ *vs.* the corresponding HFD group.

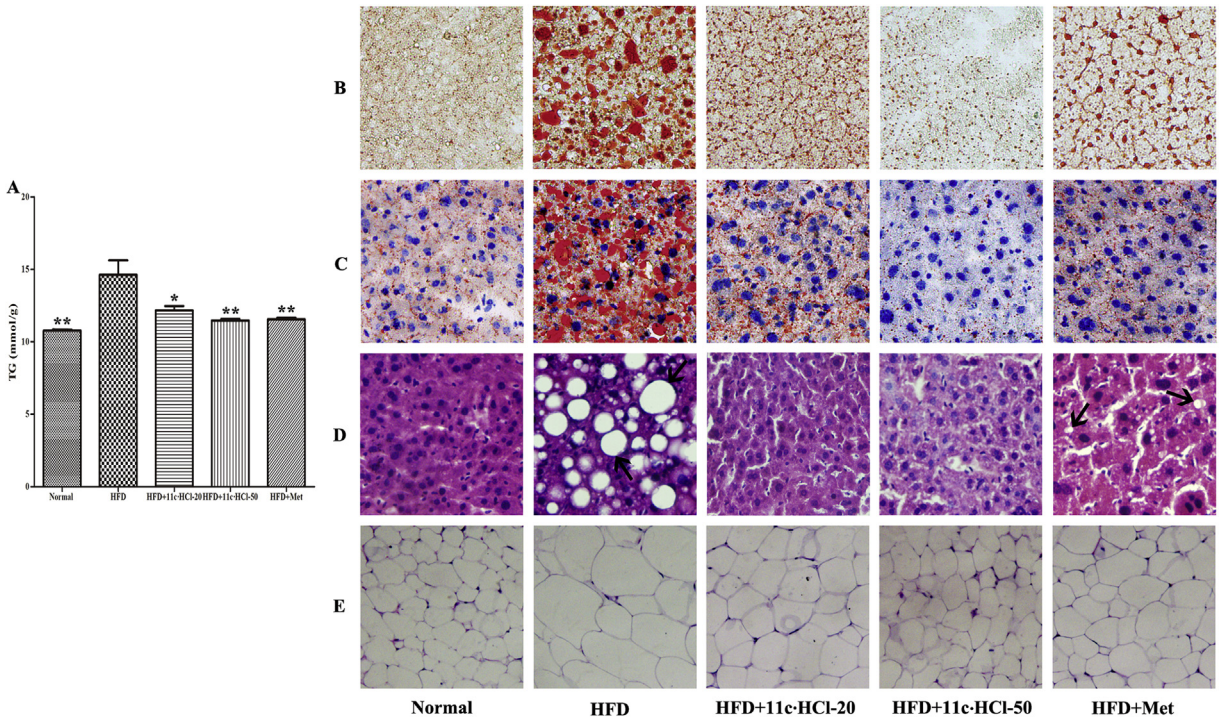


Fig. 8. (A) Effects of **11c·HCl** on hepatic TG content from DIO mice. The values represent the mean ± S.D from 6 mice in each group: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ *vs.* the corresponding HFD group. Histological examination of liver and fat (epididymal visceral adipose tissue) samples from DIO mice: (B) Oil Red O staining and (C) Oil Red O + hematoxylin staining of representative liver sections, along with H&E staining of (D) liver and (E) fat tissues (scale, 200×). Lipid droplets were marks using black arrowheads.

Q-TOF Priemier mass spectrometer (Micromass, Manchester, UK). Room temperature (rt) is within the range 20–25 °C. The purity was analyzed by HPLC system (Waters 2695, separations module) with a photodiode array detector (Waters 2996, Milford, MA, U.S.), and the chromatographic column was a reversed phase C18 column (Waters, 150 mm × 4.6 mm, i.d. 5 μm). All compounds were supplied in HPLC degree methanol with 10 μL, which was injected on a partial loop, with isocratic elution with 70% methanol and 30% water at a flow rate of 1 mL/min. The purity of all tested compounds was ≥95% according to our analytical HPLC method.

4.1.1. 2-(Methyl(pyridin-2-yl)amino)ethanol (**2**)

To a solution of 2-chloropyridine (6.0 g, 52.8 mmol) and 2-(methylamino)ethanol (11.9 g, 158.5 mmol), stirred at 150 °C overnight. After the reaction was completed, water was added into the solution. The solution was extracted with CH_2Cl_2, washed with water and brine, and dried with anhydrous Na_2SO_4. Then the solvent was removed under reduced pressure and the product appeared to be brown oil.

4.1.2. 4-(2-(Methyl(pyridin-2-yl)amino)ethoxy)benzaldehyde (**3**)

4-Fluorobenzalde (15.1 g, 121.6 mmol) was added dropwise to a stirred solution of the compound **2** (7.4 g, 48.6 mmol) in DMF (60 mL) cooled at 0 °C. Potassium tert-butoxide (6.5 g, 58.3 mmol) was added in small portions. The temperature was raised to 80 °C and stirred overnight. The mixture was quenched by water, extracted with EtOAc and combined organic phases. Then the organic phases was acidized to pH 3–4 with diluted hydrochloric acid solution, extracted with water and combined aqueous phases. The aqueous phases was basified to pH 9–10 with aqueous NaOH, extracted with EtOAc, washed with water and brine, and dried with anhydrous Na_2SO_4. Then the solvent was removed under reduced pressure. Chromatography purification of the residue product eluting with 1/5 EtOAc/PE (petroleum ether) gave the target compounds **3**.

4.1.3. 6-Amino-1-methylpyrimidine-2,4(1H,3H)-dione (**5**)

1-Methylurea (15.0 g, 202.5 mmol) and 2-cyanoacetic acid (25.8 g, 303.7 mmol) in the solvent of acetic anhydride (100 mL), were stirred at 60 °C for 5 h and the solid was formed. The precipitation was collected by filtration and washed with water to afford the important intermediate with a good yield about 65%. Dissolve the white solid (18 g, 127.5 mmol) with 20% NaOH solution (100 mL), refluxing for 2 h. After the reaction was completed, cooled down the mixture to room temperature, added the hydrochloric acid liquid to form solid. The white precipitate was collected by filtration, washed with water and ethanol to afford the products **5** without any purification.

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

To a suspension of compound **5** (10.0 g, 70.8 mmol) and DMF-DMA (25.3 g, 212.6 mmol) in DMF (50 mL) was stirred at 40 °C overnight. After the reaction was completed, water was added into the solution and cooled to room temperature. The white precipitate was collected by filtration, washed with water and ethanol to afford the products **6** without any purification.

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

To a solution of compound **6** (6.0 g, 30.6 mmol) in DMF was stirred at 0 °C for 5 min, sodium hydride (770.5 mg, 32.1 mmol) was added in small portions. Then lithium bromide (5.3 g, 61.2 mmol) was feed in the same way follow by sodium hydride. Halogenated substances (32.1 mmol) was input onetime, moved to 80 °C and stirred overnight. After the reaction was completed, poured into ice-water. And then the pure light yellow precipitate was collected by filtration, washed with water, and dried *in vacuo* to afford 89.7% of **7**.

4.1.6. General procedure for synthesis of compounds ***8***

Concentrated HCl or 28% NH_4OH was added dropwise to a stirred solution of the compound **7** (6.0 g, 19.3 mmol) in EtOH (50 mL) at ice-bath until the suspension gradually turned to clear solution. Solid was formed when heated to 40 °C about 30 min, and maintain this temperature another 5 h. The mixture was poured into ice-water and the white solid was collected by filtration, washed with water, and dried *in vacuo* to afford 87.9% of compound **8**.

4.1.7. General procedure for synthesis of compounds ***9*** *and* ***12***

Sodium nitrite (1.3 g, 19.3 mmol) was added in small portions to a stirred solution of the 5,6-diamino-1-methylpyrimidine-2,4(1*H*,3*H*)-dione derivatives (12.9 mmol) in glacial acetic acid (30 mL), the color changed from light red to blue, orange or crimson. The reaction mixture was stirred for about 5 h at ambient temperature. The colorized sediment was collected by filtration, washed with water, and dried *in vacuo* to afford 73.5% of nitrosated product. To a stirred mixture nitroso compound (2.7 g, 9.5 mmol) in 25 mL methanol, aqueous ammonia was added dropwise till the solution turn to clear. The temperature was raised to 60 °C, and sodium dithionite (3.3 g, 18.9 mmol) was added in small portion until the color disappeared. The mixture was stirred for two additional hours in order to complete the reaction. Move out the solvent under reduced pressure distillation, washed the solid with cold water, and dried under high vacuum to obtain the corresponding product.

4.1.8. General procedure for synthesis of compounds ***10*** *and* ***13***

A mixture of compound **9** or compound **12** (4.8 mmol), compound **3** (5.3 mmol) and 1.5 mL of acetic acid was refluxed for 4 h in 20 mL of ethanol. Upon cooling of the mixture, the precipitate was filtered and washed with ethanol then diethyl ether to give the Schiff-base, which was used directly in the next step. Schiff-base (3.3 mmol) was heated in 20 mL of toluene. As the mixture began to reflux, diisopropyl azodicarboxylate (6.5 mmol) was added through the condenser. After another 2 h, the reaction mixture was filtered, and the precipitate was washed with ethanol and diethyl ether to give compound **10** and **13**.

4.1.9. General procedure for synthesis of compounds ***11a–x***, ***14c***, ***15b–c***

Halogenated reagents (0.105 mmol) was added to a solution of compound **10**, **13** or **15a** (0.1 mmol) in presence of anhydrous K_2CO_3 (14.5 mg, 0.105 mmol) in DMF (3 mL). Further, the mixture was stirred at 80 °C about 5 h. The resultant mixture was extracted with EtOAc, washed with water and brine, and dried with anhydrous Na_2SO_4. Then the solvent was removed under reduced pressure to obtain the solid product. Nevertheless, compounds **14a**, **14b** and **15a** were purified by column chromatography on silica gel using 1/5 EtOAc/PE as mobile phase.

4.1.10. General procedure for synthesis of compounds ***14d***

Ethyl bromoacetate (0.3 mmol) was added to a solution of compound **13** (0.15 mmol), anhydrous K_2CO_3 (20.7 mg, 0.15 mmol) in DMF (2 mL) at room temperature. And then heat to 80 °C overnight. After the reaction was completed, the mixture was poured into water and extracted with EtOAc, washed with water and brine, and dried with anhydrous Na_2SO_4. Then the solvent was removed under reduced pressure to obtain the intermediate product. Where after the intermediate product (0.1 mmol) was dissolved in ethanol with stirring, added 1 M NaOH (200 μL), and left with stirring reflux overnight. Acetic acid (200 μL) was added to neutralize the mixture, the solvent was evaporated. The residue was poured onto ice-water, and the precipitated product was isolated by filtration, wash with water, dried.

4.1.11. General procedure for synthesis of compounds ***16a–b***

2 M NaOH (200 μL) was added to a solution of compound **15b** or **15c** (0.1 mmol) in ethanol. The mixture was heated to reflux overnight. After the reaction was completed, acetic acid (200 μL) as added to neutralize the mixture, then the solvent was evaporated. The residue was poured onto ice-water, and the precipitated product was isolated by filtration, wash with water, dried.

4.1.12. General procedure for synthesis of compound ***11c·HCl***

Sulfur dichloride (356.91 mg, 3.0 mmol) was added dropwise to a solution of compound **11c** (535.60 mg, 1.0 mmol) in dichloromethane at ice-bath. The reaction mixture was stirred for 2 h and then concentrated *in vacuo* to afford flaxen flaky solid, then drying in vacuum to get target product.

4.2. Compound analysis

*4.2.1. 2-((3-Methyl-8-(4-(2-(methyl(pyridin-2-yl)amino)ethoxy) phenyl)-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-1-yl)methyl) benzonitrile (****11a****)*

Yield 80.2%. HPLC purity: 99.28%. White solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.74 (s, 1H), 8.09 (d, J = 8.6 Hz, 3H), 7.84 (d, J = 7.6 Hz, 1H), 7.61 (t, J = 7.6 Hz, 1H), 7.51 (t, J = 7.1 Hz, 1H), 7.45 (t, J = 7.6 Hz, 1H), 7.29 (d, J = 7.9 Hz, 1H), 7.10 (d, J = 8.7 Hz, 2H), 6.66 (d, J = 8.6 Hz, 1H), 6.63–6.52 (m, 1H), 5.26 (s, 2H), 4.23 (t, J = 5.7 Hz, 2H), 3.94 (t, J = 5.7 Hz, 2H), 3.50 (s, 3H), 3.09 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 159.95, 157.89, 154.31, 151.68, 150.84, 148.17, 141.13, 137.43, 133.43, 132.82, 130.70, 127.77, 126.79, 120.41, 117.25, 114.72, 111.57, 110.46, 107.56, 105.86, 65.72, 48.39, 42.12, 37.08, 33.70, 29.53. MS(ESI), m/z: 508.29 $[M+H]^+$.

*4.2.2. 2-((3,7-Dimethyl-8-(4-(2-(methyl(pyridin-2-yl)amino) ethoxy)phenyl)-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-1-yl) methyl)benzonitrile (****11b****)*

Yield 78.9%. HPLC purity: 99.34%. White solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.10 (d, J = 4.4 Hz, 1H), 7.84 (d, J = 7.6 Hz, 1H), 7.75 (d, J = 8.2 Hz, 2H), 7.62 (t, J = 7.7 Hz, 1H), 7.52 (t, J = 7.5 Hz, 1H), 7.45 (t, J = 7.5 Hz, 1H), 7.30 (d, J = 7.8 Hz, 1H), 7.14 (d, J = 8.3 Hz, 2H), 6.68 (d, J = 8.6 Hz, 1H), 6.62–6.55 (m, 1H), 5.26 (s, 2H), 4.25 (t, J = 5.6 Hz, 2H), 3.98 (s, 3H), 3.95 (d, J = 5.4 Hz, 2H), 3.48 (s, 3H), 3.10 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 159.95, 157.89, 154.31, 151.68, 150.84, 148.17, 141.13, 137.43, 133.43, 132.82, 130.70, 127.77, 126.79, 120.41, 117.25, 114.72, 111.57, 110.46, 107.56, 105.86, 65.72, 48.39, 42.12, 37.08, 33.70, 29.53, 23.71. MS(ESI), m/z: 522.25 $[M+H]^+$.

*4.2.3. 2-((7-Ethyl-3-methyl-8-(4-(2-(methyl(pyridin-2-yl)amino) ethoxy)phenyl)-2, 6-dioxo-2,3,6,7-tetrahydro-1H-purin-1-yl) methyl)benzonitrile (****11c****)*

Yield 76.9%. HPLC purity: 99.91%. White solid. T_m: 178.0–178.3 °C; **11c·HCl**. Yield 99.46%. HPLC purity: 99.04%. Flaxen solid. T_m: 135.5–135.8 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.10 (d, J = 4.4 Hz, 1H), 7.84 (d, J = 7.6 Hz, 1H), 7.66 (d, J = 8.4 Hz, 2H), 7.61 (t, J = 7.7 Hz, 1H), 7.51 (t, J = 7.5 Hz, 1H), 7.45 (t, J = 7.5 Hz, 1H), 7.31 (d, J = 7.9 Hz, 1H), 7.15 (d, J = 8.7 Hz, 2H), 6.67 (d, J = 8.6 Hz, 1H), 6.62–6.54 (m, 1H), 5.27 (s, 2H), 4.31 (q, J = 6.9 Hz, 2H), 4.25 (t, J = 5.8 Hz, 2H), 3.96 (t, J = 5.8 Hz, 2H), 3.47 (s, 3H), 3.10 (s, 3H), 1.34 (t, J = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 159.97, 157.98, 153.95, 151.39, 150.85, 148.54, 147.48, 141.16, 137.35, 133.44, 132.85, 130.55, 127.77, 126.83, 120.60, 117.25, 114.87, 111.57, 110.40, 106.76, 105.78, 65.73, 48.38, 42.22, 41.26, 37.07, 29.55, 16.05. MS(ESI), m/z: 536.27 $[M+H]^+$.

*4.2.4. 2-((3-Methyl-8-(4-(2-(methyl(pyridin-2-yl)amino)ethoxy) phenyl)-2,6-dioxo-7-propyl-2,3,6,7-tetrahydro-1H-purin-1-yl) methyl)benzonitrile (**11d**)*

Yield 78.4%. HPLC purity: 100.00%. White solid. T_m: 144.7–145.0 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.10 (d, J = 4.2 Hz, 1H), 7.85 (d, J = 7.6 Hz, 1H), 7.65 (d, J = 8.4 Hz, 2H), 7.61 (t, J = 7.7 Hz, 1H), 7.51 (t, J = 7.6 Hz, 1H), 7.46 (t, J = 7.6 Hz, 1H), 7.30 (d, J = 7.9 Hz, 1H), 7.14 (d, J = 8.6 Hz, 2H), 6.67 (d, J = 8.6 Hz, 1H), 6.61–6.55 (m, 1H), 5.26 (s, 2H), 4.27 (t, J = 4. Hz, 2H), 4.24 (d, J = 5.6 Hz, 2H), 3.96 (t, J = 5.6 Hz, 2H), 3.48 (s, 3H), 3.10 (s, 3H), 1.71 (dd, J = 14.2, 6.9 Hz, 2H), 0.73 (t, J = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 159.89, 157.99, 154.02, 151.78, 150.84, 148.49, 147.54, 141.14, 137.32, 133.45, 132.87, 130.60, 127.76, 126.76, 120.77, 117.24, 114.84, 111.55, 110.32, 106.94, 105.73, 65.69, 48.36, 47.23, 42.23, 37.06, 29.57, 23.60, 10.54. MS(ESI), m/z: 550.29 $[M+H]^+$.

*4.2.5. 2-((7-Allyl-3-methyl-8-(4-(2-(methyl(pyridin-2-yl)amino) ethoxy)phenyl)-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-1-yl) methyl)benzonitrile (**11e**)*

Yield 71.0%. HPLC purity: 99.09%. White solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.09 (d, J = 4.0 Hz, 1H), 7.84 (d, J = 7.6 Hz, 1H), 7.68 (d, J = 8.5 Hz, 2H), 7.62 (t, J = 7.7 Hz, 1H), 7.51 (t, J = 7.7 Hz, 1H), 7.45 (t, J = 7.5 Hz, 1H), 7.29 (d, J = 7.9 Hz, 1H), 7.13 (d, J = 8.5 Hz, 2H), 6.67 (d, J = 8.6 Hz, 1H), 6.62–6.54 (m, 1H), 6.11–5.97 (m, 1H), 5.25 (s, 2H), 5.19 (d, J = 10.5 Hz, 1H), 4.97 (d, J = 4.0 Hz, 2H), 4.91 (d, J = 17.3 Hz, 1H), 4.24 (t, J = 5.6 Hz, 2H), 3.95 (t, J = 5.5 Hz, 2H), 3.49 (s, 3H), 3.09 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 160.04, 157.98, 153.89, 151.70, 150.83, 148.37, 147.53, 141.09, 137.29, 133.65, 133.40, 132.84, 130.37, 127.75, 126.78, 120.37, 117.22, 116.68, 114.78, 111.54, 110.39, 106.98, 105.70, 65.72, 48.35, 47.95, 42.15, 37.05, 29.57. MS(ESI), m/z: 548.28 $[M+H]^+$.

*4.2.6. 2-((7-Butyl-3-methyl-8-(4-(2-(methyl(pyridin-2-yl)amino) ethoxy)phenyl)-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-1-yl) methyl)benzonitrile (**11f**)*

Yield 70.0%. HPLC purity: 98.74%. White solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.10 (d, J = 4.0 Hz, 1H), 7.85 (d, J = 7.6 Hz, 1H), 7.66 (d, J = 8.4 Hz, 2H), 7.62 (t, J = 7.7 Hz, 1H), 7.51 (t, J = 7.0 Hz, 1H), 7.46 (t, J = 7.5 Hz, 1H), 7.29 (d, J = 7.9 Hz, 1H), 7.14 (d, J = 8.6 Hz, 2H), 6.67 (d, J = 8.6 Hz, 1H), 6.61–6.55 (m, 1H), 5.27 (s, 2H), 4.31 (t, J = 11.2 Hz, 2H), 4.25 (t, J = 5.7 Hz, 2H), 3.95 (t, J = 5.6 Hz, 2H), 3.47 (s, 3H), 3.10 (s, 3H), 1.66 (dd, J = 14.2, 7.3 Hz, 2H), 1.13 (dd, J = 14.5, 7.2 Hz, 2H), 0.75 (t, J = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 159.89, 157.97, 154.01, 151.78, 150.83, 148.51, 147.57, 141.13, 137.35, 133.45, 132.88, 130.60, 127.77, 126.75, 120.76, 117.23, 114.84, 111.56, 110.28, 106.91, 105.77, 65.70, 48.37, 45.57, 42.23, 37.07, 32.13, 29.56, 18.88, 13.18. MS(ESI), m/z: 504.31 $[M+H]^+$.

*4.2.7. 2-((7-Isobutyl-3-methyl-8-(4-(2-(methyl(pyridin-2-yl) amino)ethoxy)phenyl)-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-1-yl) methyl)benzonitrile (**11g**)*

Yield 71.2%. HPLC purity: 98.57%. White solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.10 (d, J = 4.1 Hz, 1H), 7.85 (d, J = 7.7 Hz, 1H), 7.67 (d, J = 8.5 Hz, 2H), 7.62 (t, J = 7.7 Hz, 1H), 7.51 (t, J = 7.6 Hz, 1H), 7.46 (t, J = 7.6 Hz, 1H), 7.28 (d, J = 7.9 Hz, 1H), 7.28 (d, J = 7.9 Hz, 1H), 7.13 (d, J = 8.5 Hz, 2H), 6.67 (d, J = 8.6 Hz, 1H), 6.61–6.53 (m, 1H), 5.26 (s, 2H), 4.23 (d, J = 6.1 Hz, 2H), 4.19 (t, J = 5.6 Hz, 2H), 3.95 (t, J = 5.6 Hz, 2H), 3.48 (s, 3H), 3.10 (s, 3H), 1.90 (m, 1H), 0.63 (d, J = 6.5 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 159.76, 157.98, 154.15, 152.34, 150.83, 148.50, 147.50, 141.12, 137.3, 133.44, 132.89, 130.75, 127.75, 126.69, 121.16, 117.22, 114.76, 111.54, 110.27, 107.04, 105.73, 65.65, 52.54, 48.38, 42.26, 37.05, 29.59, 29.15, 19.18. MS(ESI), m/z: 564.33 $[M+H]^+$.

*4.2.8. 2-((3-Methyl-8-(4-(2-(methyl(pyridin-2-yl)amino)ethoxy) phenyl)-2,6-dioxo-7-pentyl-2,3,6,7-tetrahydro-1H-purin-1-yl) methyl)benzonitrile (**11h**)*

Yield 74.7%. HPLC purity: 99.13%. White solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.10 (d, J = 4.4 Hz, 1H), 7.84 (d, J = 7.6 Hz, 1H), 7.65 (d, J = 8.4 Hz, 2H), 7.61 (t, J = 7.7 Hz, 1H), 7.51 (t, J = 7.5 Hz, 1H), 7.45 (t, J = 7.5 Hz, 1H), 7.29 (d, J = 7.9 Hz, 1H), 7.14 (d, J = 8.5 Hz, 2H), 6.67 (d, J = 8.5 Hz, 1H), 6.6–6.55 (m, 1H), 5.27 (s, 2H), 4.31 (t, J = 7.1 Hz, 2H), 4.25 (t, J = 5.6 Hz, 2H), 3.95 (t, J = 5.6 Hz, 2H), 3.47 (s, 3H), 3.10 (s, 3H), 1.75–1.63 (m, 2H), 1.19–1.04 (m, 4H), 0.74 (t, J = 6.9 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 159.90, 157.96, 154.01, 151.76, 150.81, 148.49, 147.47, 141.12, 137.34, 133.41, 132.87, 130.57, 127.75, 126.73, 120.76, 117.22, 114.83, 111.55, 110.30, 106.90, 105.76, 65.71, 48.37, 45.74, 42.21, 37.06, 29.73, 29.55, 27.73, 21.33, 13.64. MS(ESI), m/z: 578.31 $[M+H]^+$.

*4.2.9. 2-((3-Methyl-8-(4-(2-(methyl(pyridin-2-yl)amino)ethoxy) phenyl)-7-(3-methylbut-2-en-1-yl)-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-1-yl)methyl)benzonitrile (**11i**)*

Yield 82.1%. HPLC purity: 98.21%. White solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.10 (d, J = 4.2 Hz, 1H), 7.85 (d, J = 7.6 Hz, 1H), 7.65 (d, J = 8.4 Hz, 2H), 7.61 (t, J = 7.7 Hz, 1H), 7.51 (t, J = 7.6 Hz, 1H), 7.46 (t, J = 7.6 Hz, 1H), 7.30 (d, J = 7.9 Hz, 1H), 7.14 (d, J = 8.6 Hz, 2H), 6.67 (d, J = 8.6 Hz, 1H), 6.61–6.55 (m, 1H), 5.26 (s, 2H), 5.24 (t, J = 7.6 Hz, 1H), 4.27 (d, J = 10.4 Hz, 2H), 4.26 (t, J = 5.6 Hz, 2H), 3.96 (t, J = 5.6 Hz, 2H), 3.48 (s, 3H), 3.10 (s, 3H), 1.63 (s, 3H), 1.52 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 159.95, 158.00, 154.11, 151.58, 150.81, 148.43, 147.53, 141.13, 137.32, 135.72, 133.43, 132.85, 130.59, 127.76, 126.81, 120.68, 119.88, 117.24, 114.82, 111.56, 110.37, 106.89, 105.72, 65.70, 48.34, 44.36, 42.19, 37.05, 29.54, 25.17, 17.80. MS(ESI), m/z: 576.31 $[M+H]^+$.

*4.2.10. 2-((7-(2-Hydroxyethyl)-3-methyl-8-(4-(2-(methyl(pyridin-2-yl)amino)ethoxy)phenyl)-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-1-yl)methyl)benzonitrile (**11j**)*

Yield 72.8%. HPLC purity: 95.73%. White solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.10 (d, J = 3.3 Hz, 1H), 7.83 (d, J = 8.0 Hz, 1H), 7.81 (d, J = 8.4 Hz, 1H), 7.62 (t, J = 7.6 Hz, 1H), 7.51 (t, J = 7.6 Hz, 1H), 7.46 (t, J = 7.4 Hz, 1H), 7.30 (d, J = 7.6 Hz, 1H), 7.12 (d, J = 8.5 Hz, 2H), 6.67 (d, J = 8.5 Hz, 1H), 6.61–6.55 (m, 1H), 5.27 (s, 2H), 4.48 (s, 2H), 4.24 (t, J = 5.6 Hz, 2H), 3.95 (t, J = 5.5 Hz, 2H), 3.77 (d, J = 5.3 Hz, 2H), 3.49 (s, 3H), 3.10 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 159.88, 158.00, 154.05, 152.75 150.83, 148.58, 147.54, 141.16, 137.31, 133.42, 132.83, 131.16, 127.78, 126.76, 120.84, 117.25, 114.58, 111.55, 110.48, 106.99, 105.73, 65.69, 62.77, 59.95, 48.37, 42.20, 37.06, 29.55. MS(ESI), m/z: 574.28 $[M+Na]^+$.

*4.2.11. Ethyl-2-(1-(2-cyanobenzyl)-3-methyl-8-(4-(2-(methyl(pyridin-2-yl)amino) ethoxy)phenyl)-2,6-dioxo-2,3-dihydro-1H-purin-7(6H)-yl)acetate (**11k**)*

Yield 85.0%. HPLC purity: 99.12%. White solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.10 (d, J = 4.0 Hz, 1H), 7.86 (d, J = 7.6 Hz, 1H), 7.67 (d, J = 8.5 Hz, 2H), 7.61 (t, J = 7.7 Hz, 1H), 7.51 (t, J = 7.1 Hz, 1H), 7.46 (t, J = 7.6 Hz, 1H), 7.31 (d, J = 7.8 Hz, 1H), 7.13 (d, J = 8.6 Hz, 2H), 6.67 (d, J = 8.5 Hz, 1H), 6.61–6.55 (m, 1H), 5.23 (s, 2H), 5.18 (s, 2H), 4.24 (t, J = 5.7 Hz, 2H), 4.06 (t, J = 5.7 Hz, 2H), 3.95 (q, J = 7.2 Hz, 2H), 3.50 (s, 3H), 3.09 (s, 3H), 1.18 (t, J = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 167.50, 160.22, 157.99, 154.25, 152.44, 150.78, 148.18, 147.53, 140.93, 137.32, 133.42, 132.85, 130.44, 127.83, 126.73, 119.88, 117.2, 115.02, 111.56, 110.48, 107.26, 105.73, 65.77, 61.55, 48.34, 47.58, 42.10, 37.06, 29.64, 13.84. MS(ESI), m/z: 594.29 $[M+H]^+$.

*4.2.12. 2-(1-(2-Cyanobenzyl)-3-methyl-8-(4-(2-(methyl(pyridin-2-yl)amino)ethoxy) phenyl)-2,6-dioxo-2,3-dihydro-1H-purin-7(6H)-yl)acetic acid (**11l**)*

Yield 57.9%. HPLC purity: 97.32%. White solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.09 (d, J = 4.0 Hz, 1H), 7.85 (d, J = 7.6 Hz, 1H), 7.67 (d, J = 8.5 Hz, 2H), 7.62 (t, J = 7.7 Hz, 1H), 7.51 (t, J = 7.1 Hz, 1H), 7.46 (t, J = 7.6 Hz, 1H), 7.31 (d, J = 7.8 Hz, 1H), 7.13 (d, J = 8.6 Hz, 2H), 6.67 (d, J = 8.5 Hz, 1H), 6.61–6.55 (m, 1H), 5.24 (s, 2H), 5.07 (s, 2H), 4.32 (t, J = 4.8 Hz, 2H), 4.07 (t, J = 4.8 Hz, 2H), 3.50 (s, 3H), 3.25 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 167.51, 160.22, 157.99, 154.25, 152.45, 150.78, 148.18, 147.52, 140.93, 137.32, 133.42, 132.85, 130.44, 127.83, 126.73, 119.88, 117.21, 115.02, 111.56, 110.48, 107.26, 105.74, 65.77, 61.55, 48.34, 42.10, 37.07, 29.64. MS(ESI), m/z: 566.29 $[M+H]^+$.

*4.2.13. Ethyl-4-(1-(2-cyanobenzyl)-3-methyl-8-(4-(2-(methyl(pyridin-2-yl)amino) ethoxy)phenyl)-2,6-dioxo-2,3-dihydro-1H-purin-7(6H)-yl)butanoate (**11m**)*

Yield 75.0%. HPLC purity: 99.10%. White solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.10 (d, J = 4.0 Hz, 1H), 7.85 (d, J = 7.6 Hz, 1H), 7.67 (d, J = 8.5 Hz, 2H), 7.62 (t, J = 7.7 Hz, 1H), 7.51 (t, J = 7.1 Hz, 1H), 7.46 (t, J = 7.6 Hz, 1H), 7.31 (d, J = 7.8 Hz, 1H), 7.13 (d, J = 8.6 Hz, 2H), 6.67 (d, J = 8.5 Hz, 1H), 6.61–6.55 (m, 1H), 5.27 (s, 2H), 4.37 (t, J = 6.6 Hz, 2H), 4.24 (t, J = 5.6 Hz, 2H), 3.96 (t, J = 5.6 Hz, 2H), 3.88 (q, J = 7.2 Hz, 2H), 3.48 (s, 3H), 3.10 (s, 3H), 2.19 (t, J = 6.8 Hz, 2H), 1.84 (m, 2H), 1.06 (t, J = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.80, 159.93, 157.96, 154.06, 151.88, 150.82, 148.55, 147.47, 141.10, 137.35, 133.40, 132.85, 130.71, 127.77, 126.75, 120.57, 117.23, 114.78, 111.56, 110.35, 106.96, 105.77, 65.71, 59.86, 48.38, 45.12, 42.21, 37.08, 30.01, 29.55, 25.29, 13.95. MS(ESI), m/z: 622.27 $[M+H]^+$.

*4.2.14. 4-(1-(2-Cyanobenzyl)-3-methyl-8-(4-(2-(methyl(pyridin-2-yl)amino)ethoxy) phenyl)-2,6-dioxo-2,3-dihydro-1H-purin-7(6H)-yl)butanoic acid (**11n**)*

Yield 50.6%. HPLC purity: 96.32%. White solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.09 (d, J = 4.0 Hz, 1H), 7.86 (d, J = 7.6 Hz, 1H), 7.67 (d, J = 8.5 Hz, 2H), 7.63 (t, J = 7.7 Hz, 1H), 7.51 (t, J = 7.1 Hz, 1H), 7.46 (t, J = 7.6 Hz, 1H), 7.32 (d, J = 7.8 Hz, 1H), 7.13 (d, J = 8.6 Hz, 2H), 6.67 (d, J = 8.5 Hz, 1H), 6.62–6.55 (m, 1H), 5.25 (s, 2H), 4.37 (t, J = 5.6 Hz, 2H), 4.24 (t, J = 5.6 Hz, 2H), 4.07 (t, J = 6.6 Hz, 2H), 2.37 (t, J = 6.8 Hz, 2H), 2.19 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.80, 159.93, 157.96, 154.06, 151.88, 150.82, 148.55, 147.47, 141.10, 137.35, 133.40, 132.85, 130.71, 127.77, 126.75, 120.57, 117.23, 114.78, 111.56, 110.35, 106.96, 105.77, 65.71, 59.86, 48.38, 42.21, 37.08, 30.01, 29.55, 25.29. MS(ESI), m/z: 594.29 $[M+H]^+$.

*4.2.15. 1-(4-Methoxybenzyl)-3-methyl-8-(4-(2-(methyl(pyridin-2-yl)amino)ethoxy) phenyl)-1H-purine-2,6(3H,7H)-dione (**11o**)*

Yield 89.1%. HPLC purity: 98.90%. White solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.66 (s, 1H), 8.08 (d, J = 4.0 Hz, 2H), 7.96 (d, J = 7.6 Hz, 1H), 7.51 (t, J = 7.0 Hz, 1H), 7.27 (d, J = 8.6 Hz, 2H), 7.08 (d, J = 8.8 Hz, 2H), 6.85 (d, J = 8.6 Hz, 2H), 6.66 (d, J = 8.0 Hz, 1H), 6.60–6.55 (m, 1H), 5.02 (s, 2H), 4.22 (t, J = 5.8 Hz, 2H), 3.94 (t, J = 5.8 Hz, 2H), 3.71 (s, 3H), 3.49 (s, 3H), 3.09 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 160.05, 158.33, 157.99, 153.82, 150.94, 150.16, 147.52, 137.29, 129.76, 129.12, 128.10, 121.20, 114.77, 113.57, 111.54, 105.70, 65.66, 54.99, 48.36, 43.06, 37.03, 29.71. MS(ESI), m/z: 513.29 $[M+H]^+$.

*4.2.16. 7-Ethyl-1-(4-methoxybenzyl)-3-methyl-8-(4-(2-(methyl(pyridin-2-yl)amino) ethoxy)phenyl)-1H-purine-2,6(3H,7H)-dione (**11p**)*

Yield 83.6%. HPLC purity: 98.76%. White solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.09 (d, J = 4.7 Hz, 1H), 7.63 (d, J = 8.6 Hz, 2H), 7.52 (t, J = 7.8 Hz, 1H), 7.28 (d, J = 8.4 Hz, 2H), 7.13 (d, J = 8.6 Hz, 2H), 6.86 (d, J = 8.5 Hz, 2H), 6.68 (d, J = 8.2 Hz, 1H), 6.64–6.55 (m, 1H), 5.02 (s, 2H), 4.31 (q, J = 7.2 Hz, 2H), 4.24 (t, J = 5.8 Hz, 2H), 3.95 (t, J = 5.7 Hz, 2H), 3.71 (s, 3H), 3.45 (s, 3H), 3.10 (s, 3H), 1.34 (t, J = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 159.88, 158.36, 157.84, 154.00, 151.17, 150.80, 148.15, 147.24, 137.46, 130.52, 129.68, 129.22, 120.68, 114.80, 113.59, 111.56, 106.72, 105.89, 65.69, 55.00, 48.41, 43.04, 41.17, 37.07, 29.43, 16.06.MS(ESI), m/z: 541.24 $[M+H]^+$.

*4.2.17. 7-Allyl-1-(4-methoxybenzyl)-3-methyl-8-(4-(2-(methyl(pyridin-2-yl)amino) ethoxy)phenyl)-1H-purine-2,6(3H,7H)-dione (**11q**)*

Yield 84.1%. HPLC purity: 98.82%. White solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.09 (d, J = 4.4 Hz, 1H), 7.65 (d, J = 8.4 Hz, 2H), 7.51 (t, J = 7.9 Hz, 1H), 7.27 (d, J = 8.3 Hz, 2H), 7.11 (d, J = 8.5 Hz, 2H), 6.85 (d, J = 8.3 Hz, 2H), 6.66 (d, J = 8.6 Hz, 1H), 6.61–6.55 (m, 1H), 6.09–5.98 (m, 1H), 5.18 (d, J = 10.6 Hz, 1H), 4.99 (s, 2H), 4.98 (d, J = 4.0 Hz, 2H), 4.89 (d, J = 17.3 Hz, 1H), 4.23 (t, J = 5.6 Hz, 2H), 3.94 (t, J = 5.7 Hz, 2H), 3.71 (s, 3H), 3.47 (s, 3H), 3.09 (s, 3H). ^{13}C NMR (100 MHz, DMSO d_6): δ 159.97, 158.37, 157.98, 153.97, 151.51, 150.79, 147.99, 147.52, 137.30, 133.76, 130.35, 129.63, 129.24, 120.46, 116.51, 114.74, 113.57, 111.54, 106.93, 105.71, 65.70, 54.99, 48.35, 47.86, 43.0, 37.04, 29.45. MS(ESI), m/z: 553.31 $[M+H]^+$.

*4.2.18. 7-Ethyl-1-(4-fluorobenzyl)-3-methyl-8-(4-(2-(methyl(pyridin-2-yl)amino) ethoxy)phenyl)-1H-purine-2,6(3H,7H)-dione (**11r**)*

Yield 78.0%. HPLC purity: 98.78%. White solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.09 (d, J = 4.7 Hz, 1H), 7.64 (d, J = 8.6 Hz, 2H), 7.51 (t, J = 7.3 Hz, 1H), 7.41 (d, J = 8.7 Hz, 2H), 7.13 (t, J = 8.0 Hz, 4H), 6.67 (d, J = 8.5 Hz, 1H), 6.61–6.55 (m, 1H), 5.07 (s, 2H), 4.31 (q, J = 7.2 Hz, 2H), 4.24 (t, J = 5.8 Hz, 2H), 3.95 (t, J = 5.7 Hz, 2H), 3.46 (s, 3H), 3.09 (s, 3H), 1.34 (t, J = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 160.03, 157.99, 153.95, 151.60, 150.82, 148.11, 147.53, 137.30, 130.37, 129.77, 129.69, 120.44, 115.09, 114.88, 114.76, 111.55, 106.94, 105.72, 65.70, 55.00, 48.35, 47.89, 42.93, 37.05, 29.49. MS(ESI), m/z: 529.25 $[M+H]^+$.

*4.2.19. 7-Allyl-1-(4-fluorobenzyl)-3-methyl-8-(4-(2-(methyl(pyridin-2-yl)amino)etoxy) phenyl)-1H-purine-2,6(3H,7H)-dione (**11s**)*

Yield 73.9%. HPLC purity: 98.34%. White solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.09 (d, J = 4.6 Hz, 1H), 7.66 (d, J = 8.4 Hz, 2H), 7.51 (t, J = 7.9 Hz, 1H), 7.19 (d, J = 8.7 Hz, 2H), 7.12 (d, J = 7.9 Hz, 2H), 6.84 (d, J = 8.3 Hz, 2H), 6.66 (d, J = 8.6 Hz, 1H), 6.60–6.55 (m, 1H), 6.09–5.97 (m, 1H), 5.27 (d, J = 10.6 Hz, 1H), 5.04 (d, J = 7.2 Hz, 2H), 4.90 (d, J = 17.2 Hz, 1H), 4.50 (s, 2H), 4.23 (t, J = 5.7 Hz, 2H), 3.94 (t, J = 5.6 Hz, 2H), 3.48 (s, 3H), 3.09 (s, 3H). ^{13}C NMR (100 MHz, DMSO d_6): δ 160.03, 157.99, 153.95, 151.60, 150.82, 148.11, 147.53, 137.30, 133.73, 130.37, 129.77, 129.69, 120.44, 116.56, 115.09, 114.88, 114.76, 111.55, 106.94, 105.72, 65.70, 48.35, 47.89, 42.93, 37.05, 29.49. MS(ESI), m/z: 541.25 $[M+H]^+$.

*4.2.20. 1-Allyl-3-methyl-8-(4-(2-(methyl(pyridin-2-yl)amino) ethoxy)phenyl)-1H-purine-2,6(3H,7H)-dione (**11t**)*

Yield 87.9%. HPLC purity: 98.71%. White solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.60 (s, 1H), 8.12 (d, J = 4.0 Hz, 1H), 8.00 (d, J = 8.4 Hz, 2H), 7.50 (d, J = 6.8 Hz, 1H), 7.08 (d, J = 8.8 Hz, 2H), 6.66 (d, J = 8.6 Hz, 1H), 6.63–6.56 (m, 1H), 5.92–5.81 (m, 1H), 5.10 (d, J = 5.6 Hz, 1H), 5.06 (d, J = 5.6 Hz, 1H), 4.50 (d, J = 4.8 Hz, 2H), 4.22 (t, J = 5.8 Hz, 2H), 3.94 (t, J = 5.8 Hz, 2H), 3.50 (s, 3H), 3.09 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 160.07, 158.00, 153.52, 150.72, 150.08, 148.79, 147.53, 137.32, 133.16, 128.12, 121.22, 115.98, 114.83, 111.55, 107.11, 105.73, 65.67, 48.37, 42.52, 37.04, 29.72. MS(ESI), m/z: 433.32 $[M+H]^+$.

*4.2.21. 1,7-Diallyl-3-methyl-8-(4-(2-(methyl(pyridin-2-yl)amino) ethoxy)phenyl)-1H-purine-2,6(3H,7H)-dione (**11u**)*

Yield 79.2%. HPLC purity: 95.34%. White solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.15 (d, J = 4.0 Hz, 1H), 7.71 (d, J = 8.4 Hz, 2H), 7.56 (t, J = 7.5 Hz, 1H), 7.17 (d, J = 8.5 Hz, 2H), 6.72 (d, J = 8.6 Hz, 1H), 6.66–6.58 (m, 1H), 6.15–6.03 (m, 1H), 5.91–5.81 (m, 1H), 5.23 (d, J = 10.4 Hz, 1H), 5.16 (d, J = 10.4 Hz, 1H), 5.12 (d, J = 17.2 Hz, 1H), 5.02 (d, J = 4.4 Hz, 2H), 4.94 (d, J = 17.2 Hz, 1H), 4.54 (d, J = 4.4 Hz, 2H), 4.28 (t, J = 5.6 Hz, 2H), 4.00 (t, J = 5.6 Hz, 2H), 3.53 (s, 3H), 3.14 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 159.96, 157.98, 153.63, 151.39, 150.48, 147.99, 147.52, 137.28, 133.73, 132.97, 130.30, 120.46, 116.50, 116.24, 114.73, 111.54, 106.86, 105.70, 65.69, 48.35, 47.84, 42.42, 37.04, 29.41. MS(ESI), m/z: 473.27 $[M+H]^+$.

*4.2.22. 3-Methyl-8-(4-(2-(methyl(pyridin-2-yl)amino)ethoxy) phenyl)-1-propyl-1H-purine-2,6(3H,7H)-dione (**11v**)*

Yield 86.9%. HPLC purity: 97.22%. White solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.60 (s, 1H), 8.09 (d, J = 3.9 Hz, 1H), 8.04 (d, J = 8.3 Hz, 2H), 7.51 (t, J = 7.8 Hz, 1H), 7.08 (d, J = 8.7 Hz, 2H), 6.66 (d, J = 8.5 Hz, 1H), 6.57 (s, 1H), 4.22 (d, J = 5.5 Hz, 2H), 3.94 (d, J = 5.5 Hz, 2H), 3.86 (t, J = 6.7 Hz, 2H), 3.49 (s, 3H), 3.09 (s, 3H), 1.58 (m, 2H), 0.88 (t, J = 7.5 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 160.04, 158.00, 153.88, 150.94, 149.96, 148.64, 147.53, 137.32, 128.09, 121.25, 114.82, 111.55, 107.18, 105.73, 65.67, 48.37, 42.10, 37.04, 29.66, 20.82, 11.16. MS(ESI), m/z: 435.27 $[M+H]^+$.

*4.2.23. 7-Ethyl-3-methyl-8-(4-(2-(methyl(pyridin-2-yl)amino) ethoxy)phenyl)-1-propyl-1H-purine-2,6(3H,7H)-dione (**11w**)*

Yield 82.7%. HPLC purity: 99.20%. White solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.16 (d, J = 3.9 Hz, 1H), 7.69 (d, J = 8.3 Hz, 2H), 7.57 (t, J = 7.7 Hz, 1H), 7.19 (d, J = 8.3 Hz, 2H), 6.73 (d, J = 8.6 Hz, 1H), 6.64 (t, J = 5.7 Hz, 1H), 4.36 (q, J = 7.2 Hz, 2H), 4.30 (d, J = 5.5 Hz, 2H), 4.01 (t, J = 5.5 Hz, 2H), 3.92 (t, J = 7.2 Hz, 2H), 3.51 (s, 3H), 3.16 (s, 3H), 1.64 (m, 2H), 1.40 (t, J = 6.8 Hz, 3H), 0.94 (t, J = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 159.86, 157.96, 154.04, 150.96, 150.71, 148.01, 147.46, 137.34, 130.51, 120.72, 114.80, 111.55, 106.69, 105.77, 65.69, 48.37, 42.00, 41.12, 37.06, 29.34, 20.81, 16.05, 11.15. MS(ESI), m/z: 463.29 $[M+H]^+$.

*4.2.24. 3-Methyl-8-(4-(2-(methyl(pyridin-2-yl)amino)ethoxy) phenyl)-1,7-dipropyl-1H-purine-2,6(3H,7H)-dione (**11x**)*

Yield 79.5%. HPLC purity: 98.35%. White solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.15 (d, J = 3.9 Hz, 1H), 7.68 (d, J = 8.4 Hz, 2H), 7.57 (t, J = 7.8 Hz, 1H), 7.18 (d, J = 8.4 Hz, 2H), 6.73 (d, J = 8.6 Hz, 1H), 6.63 (t, J = 5.8 Hz, 1H), 4.33 (t, J = 7.2 Hz, 2H), 4.28 (t, J = 5.6 Hz, 2H), 4.01 (t, J = 5.6 Hz, 2H), 3.92 (t, J = 7.2 Hz, 2H), 3.51 (s, 3H), 3.15 (s, 3H), 1.75 (m, 2H), 1.63 (m 2H), 0.93 (t, J = 7.4 Hz, 3H), 0.78 (t, J = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 59.79, 157.99, 154.12, 151.37, 147.95, 147.52, 137.29, 130.55, 120.90, 114.77, 111.54, 106.89, 105.71, 65.68, 48.37, 47.22, 41.99, 37.04, 29.34, 23.61, 20.80, 11.13, 10.49. S(ESI), m/z: 477.27 $[M+H]^+$.

*4.2.25. 3-Methyl-8-(4-(2-(methyl(pyridin-2-yl)amino)ethoxy) phenyl)-1H-purine-2,6 (3H,7H)-dione (**13**)*

Yield 78.0%. HPLC purity: 96.44%. White solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.59 (s, 1H), 11.08 (s, 1H), 8.09 (d, J = 4.5 Hz, 1H), 8.05 (d, J = 8.4 Hz, 2H), 7.51 (t, J = 7.7 Hz, 1H), 7.07 (d, J = 8.5 Hz, 2H), 6.66 (d, J = 8.6 Hz, 1H), 6.60–6.55 (m, 1H), 4.22 (t, J = 5.6 Hz, 2H), 3.94 (t, J = 5.6 Hz, 2H), 3.42 (s, 3H), 3.09 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 162.28, 159.97, 157.99, 156.34, 154.33, 151.25, 151.11, 147.53, 137.31, 127.99, 121.31, 114.80, 111.54, 105.72, 75.19, 65.65, 48.37, 37.03. MS(ESI), m/z: 410.14 $[M+Na]^+$.

*4.2.26. 7-Ethyl-3-methyl-8-(4-(2-(methyl(pyridin-2-yl)amino) ethoxy)phenyl)-1H-purine-2,6(3H,7H)-dione (**14a**)*

Yield 68.3%. HPLC purity: 97.32%. White solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.13 (s, 1H), 8.09 (d, J = 4.5 Hz, 1H), 7.63 (d, J = 8.7 Hz, 2H), 7.51 (t, J = 7.0 Hz, 1H), 7.13 (d, J = 8.7 Hz, 2H), 6.67 (d, J = 8.6 Hz, 1H), 6.60–6.55 (m, 1H), 4.36 (d, J = 6.9 Hz, 2H), 4.30 (d, J = 5.9 Hz, 2H), 3.95 (t, J = 5.9 Hz, 2H), 3.38 (s, 2H), 3.09 (s, 3H), 1.33 (t, J = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 159.82, 158.00, 154.56, 149.92, 150.83, 149.57, 147.53, 137.30, 130.47, 120.77, 114.78, 111.55, 107.13, 105.72, 65.69, 48.36, 41.08, 37.05, 28.40, 16.03. MS(ESI), m/z: 421.25 $[M+H]^+$.

*4.2.27. 3-Methyl-8-(4-(2-(methyl(pyridin-2-yl)amino)ethoxy) phenyl)-7-(3-methylbut-2-en-1-yl)-1H-purine-2,6(3H,7H)-dione (**14b**)*

Yield 64.9%. HPLC purity: 97.81%. White solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.11 (s, 1H), 8.09 (d, J = 4.4 Hz, 1H), 7.62 (d, J = 8.6 Hz, 2H), 7.51 (t, J = 7.8 Hz, 1H), 7.12 (d, J = 8.6 Hz, 2H), 6.66 (d, J = 8.5 Hz, 1H), 6.62–6.53 (m, 1H), 5.22 (t, J = 7.6 Hz, 1H), 4.92 (d, J = 5.5 Hz, 2H), 4.23 (d, J = 5.6 Hz, 2H), 3.95 (d, J = 5.5 Hz, 3H), 3.38 (s, 3H), 3.09 (s, 2H), 1.63 (s, 3H), 1.53 (s, 3H). ^{13}C NMR (1001 MHz, DMSO-d_6): δ 159.80, 158.00, 154.71, 151.00, 150.84, 149.45, 147.53, 137.32, 135.67, 130.51, 120.85, 119.93, 114.74, 111.56, 107.26, 105.72, 65.67, 48.34, 44.16, 37.04, 28.40, 25.19, 17.76. MS(ESI), m/z: 461.27 $[M+H]^+$.

*4.2.28. 1,7-Diethyl-3-methyl-8-(4-(2-(methyl(pyridin-2-yl)amino) ethoxy)phenyl)-1H-purine-2,6(3H,7H)-dione (**14c**)*

Yield 82.5%. HPLC purity: 95.09%. White solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.09 (d, J = 3.6 Hz, 1H), 7.63 (d, J = 8.6 Hz, 2H), 7.52 (t, J = 7.2 Hz, 1H), 7.13 (d, J = 8.6 Hz, 2H), 6.67 (d, J = 8.6 Hz, 1H), 6.62–6.56 (m, 1H), 4.30 (q, J = 6.7 Hz, 2H), 4.24 (t, J = 5.6 Hz, 2H), 3.96 (t, J = 5.6 Hz, 2H), 3.92 (q, J = 6.7 Hz, 2H), 3.45 (s, 3H), 3.10 (s, 3H), 1.34 (t, J = 6.9 Hz, 3H), 1.14 (t, J = 6.9 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 159.86, 158.00, 153.82, 150.94, 150.52, 148.00, 147.53, 137.30, 130.50, 120.72, 114.80, 111.55, 106.75, 105.72, 65.70, 48.36, 41.11, 37.05, 35.61, 29.31, 16.06, 13.11. MS(ESI), m/z: 449.25 $[M+H]^+$.

*4.2.29. 2,2′-(3-Methyl-8-(4-(2-(methyl(pyridin-2-yl)amino) ethoxy)phenyl)-2,6-dioxo-2,3-dihydro-1H-purine-1,7(6H)-diyl) diacetic acid (**14d**)*

Yield 73.8%. HPLC purity: 96.03%. White solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.07 (d, J = 4.0 Hz, 1H), 7.68 (t, J = 7.7 Hz, 1H), 7.60 (d, J = 8.1 Hz, 2H), 7.12 (d, J = 7.8 Hz, 2H), 6.91 (d, J = 8.6 Hz, 1H), 6.70–6.65 (m, 1H), 5.07 (s, 2H), 4.53 (s, 2H), 4.27 (t, J = 5.6 Hz, 2H), 4.00 (t, J = 5.6 Hz, 2H), 3.49 (s, 3H), 3.16 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.43, 168.86, 160.00, 158.00, 153.85, 152.34, 150.53, 148.03, 147.89, 137.32, 130.40, 120.17, 114.97, 111.72, 107.12, 105.72, 65.58, 48.91, 47.51, 41.68, 37.43, 29.48. MS(ESI), m/z: 531.13 $[M+Na]^+$.

*4.2.30. (3-Methyl-8-(4-(2-(methyl(pyridin-2-yl)amino)ethoxy) phenyl)-2,6-dioxo-2,3-dihydro-1H-purin-7(6H)-yl)methyl pivalate (**15a**)*

Yield 47.3%. HPLC purity: 97.21%. White solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.27 (s, 1H), 8.09 (d, J = 4.0 Hz, 1H), 7.67 (d, J = 8.7 Hz, 2H), 7.51 (t, J = 7.8 Hz, 1H), 7.15 (d, J = 8.7 Hz, 2H), 6.66 (d, J = 8.5 Hz, 1H), 6.63–6.55 (m, 1H), 6.14 (s, 2H), 4.24 (t, J = 5.8 Hz, 2H), 3.95 (t, J = 5.8 Hz, 2H), 3.40 (s, 3H), 3.09 (s, 3H), 1.08 (s, 9H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 177.90, 159.90, 157.99, 154.39, 151.12, 150.25, 149.97, 147.52, 137.29, 130.36, 121.49, 114.75, 111.53, 107.81, 105.70, 65.64, 64.53, 61.47, 48.37, 41.70, 37.03, 28.72. MS(ESI), m/z: 529.29 $[M+Na]^+$.

*4.2.31. (1-(2-Ethoxy-2-oxoethyl)-3-methyl-8-(4-(2-(methyl(pyridin-2-yl)amino)ethoxy) phenyl)-2,6-dioxo-2,3-dihydro-1H-purin-7(6H)-yl)methyl pivalate (**15b**)*

Yield 60.2%. HPLC purity: 98.73%. White solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.10 (d, J = 4.0 Hz, 1H), 7.70 (d, J = 8.8 Hz, 2H), 7.59 (d, J = 8.8 Hz, 1H), 7.17 (d, J = 8.5 Hz, 2H), 6.66 (d, J = 8.6 Hz, 1H), 6.61–6.55 (m, 1H), 6.16 (s, 2H), 4.63 (s, 2H), 4.25 (t, J = 5.8 Hz, 2H), 4.14 (q, J = 7.1 Hz, 2H), 3.95 (t, J = 5.8 Hz, 2H), 3.50 (s, 3H), 3.09 (s, 3H), 1.20 (t, J = 7.1 Hz, 3H), 1.08 (s, 9H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 177.92, 167.48, 160.23, 157.99, 153.74, 152.55, 150.47, 148.02, 147.53, 137.30, 130.46, 119.81, 114.99, 111.55, 107.02, 105.72, 65.76, 64.53, 61.57, 60.96, 48.34, 47.56, 41.71, 37.05, 29.52, 13.91. MS(ESI), m/z: 593.3 $[M+H]^+$.

*4.2.32. Ethyl-4-(3-methyl-8-(4-(2-(methyl(pyridin-2-yl)amino) ethoxy)phenyl)-2,6-dioxo-7-((pivaloyloxy)methyl)-2,3,6,7-tetrahydro-1H-purin-1-yl)butanoate (**15c**)*

Yield 56.9%. HPLC purity: 98.92%. White solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.09 (d, J = 4.0 Hz, 1H), 7.67 (d, J = 8.4 Hz, 2H), 7.51 (t, J = 7.7 Hz, 1H), 7.15 (d, J = 8.5 Hz, 2H), 6.66 (d, J = 8.6 Hz, 1H), 6.61–6.55 (m, 1H), 6.16 (s, 2H), 4.25 (t, J = 5.6 Hz, 2H), 4.01 (t, J = 5.6 Hz, 2H), 3.97 (t, J = 6.6 Hz, 2H), 3.90 (q, J = 7.2 Hz, 2H), 3.46 (s, 3H), 3.09 (s, 3H), 2.34 (t, J = 6.8 Hz, 2H), 1.89 (m, 2H), 1.16 (t, J = 7.1 Hz, 3H), 1.11 (s, 9H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 177.92, 173.62, 159.51, 153.94, 152.06, 150.96, 149.71, 148.34, 143.25, 136.76, 128.06, 121.58, 114.76, 112.30, 112.03, 107.29, 65.27, 64.53, 61.47, 59.86, 49.39, 43.21, 38.47, 37.03, 31.17, 29.69, 13.92. MS(ESI), m/z: 643.31 $[M+Na]^+$.

*4.2.33. 2-(3-Mmethyl-8-(4-(2-(methyl(pyridin-2-yl)amino)ethoxy) phenyl)-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-1-yl)acetic acid (**16a**)*

Yield 68.4%. HPLC purity: 97.63%. White solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.73 (s, 1H), 12.92 (s, 1H), 8.08 (d, J = 4.2 Hz, 1H), 7.70 (d, J = 8.4 Hz, 2H), 7.58 (t, J = 7.7 Hz, 1H), 7.08 (d, J = 8.5 Hz, 2H), 6.77 (d, J = 8.6 Hz, 1H), 6.61–6.65 (m, 1H), 4.56 (s, 2H), 4.24 (t, J = 5.4 Hz, 2H), 3.96 (t, J = 5.4 Hz, 2H), 3.51 (s, 3H), 3.11 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.92, 159.51, 153.93, 152.06, 150.96, 149.71, 148.54, 143.25, 136.76, 128.06, 121.58, 114.76, 112.30, 112.03, 107.29, 65.27, 50.39, 37.07, 29.69, 23.08. MS(ESI), m/z: 451.29 $[M+H]^+$.

*4.2.34. 4-(3-Methyl-8-(4-(2-(methyl(pyridin-2-yl)amino)ethoxy) phenyl)-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-1-yl)butanoic acid (**16b**)*

Yield 50.9%. HPLC purity: 98.51%. White solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.73 (s, 1H), 11.02 (s, 1H), 8.08 (d, J = 4.0 Hz, 1H), 7.73 (d, J = 8.4 Hz, 2H), 7.58 (t, J = 7.7 Hz, 1H), 7.08 (d, J = 8.8 Hz, 2H), 6.77 (d, J = 8.6 Hz, 1H), 6.61–6.65 (m, 1H), 4.24 (t, J = 5.5 Hz, 2H), 3.96 (t, J = 5.4 Hz, 2H), 3.86 (t, J = 5.5 Hz,2H), 3.51 (s, 3H), 3.11 (s, 3H). 2.27 (t, J = 5.5 Hz, 2H), 1.86 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.62, 159.51, 153.94, 152.06, 150.96, 149.71, 148.34, 143.25, 136.76, 128.06, 121.58, 114.76, 112.30, 112.03, 107.29, 65.27, 59.86, 49.39, 43.21, 37.07, 29.69, 23.58. MS(ESI), m/z:479.27 $[M+H]^+$.

4.3. Biological evaluation

4.3.1. Determination the content of TG in 3T3-L1 adipocytes

Murine 3T3-L1 preadipocytes (ATCC, Rockville, MD) were plated and grown to 2 days after confluence in 96-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 ethyl-3-isobutylxantine (IBMX), 0.25 μM dexamethasone (DEX), and 10 μg/mL insulin. The medium was again changed to serum-containing DMEM that contained 10 μg/mL insulin but no IBMX or DEX every two days until all the preadipocytes differentiated successfully. Generally full differentiation needs 8–12 days. The twelfth day after differentiation, the culture medium was replaced by DMEM supplemented with compounds (10 μM), RSG (10 μM), Met (1.0 mM), and AICAR (0.5 mM). After 24 h, wash the 3T3-L1 cell with precooling PBS twice and then drying, join 50 Mm KOH liquor in cracking reaction under 60 °C, 10 min later collecting the supernatant after centrifugation. The determination of TG was performed by commercial Triglyceride Assay Kit (Biovision, San Francisco Bay, USA.). The results were recorded for three independent experiments. The TG inhibition ratio was calculated using $(1 - TG_{model}/TG_{compound}) \times 100\%$.

4.3.2. Animal model and treatment of DIO mice

C57BL/6J mice were obtained from Beijing HFK Bioscience CO., LTD and housed individually in a room maintained at 25 °C on a light/dark schedule. For DIO mice, 3-week-old male C57BL/6J mice were fed a high fat diet (HFD, Research Diets, D12492) for 12 weeks, thus their weight is higher 20% in the normal group. Then these animals were assigned to five groups randomly consisting of 6 mice each. The mice received a normal diet with 18.94% of energy derived from fat, 31.67% from protein, and 49.39% from carbohydrates and received a high fat diet with 60.0% of calories from fat, 20.0% from protein, and 20.0% from carbohydrates. HFD + **11c** (50 mg/kg/day), HFD + **11d** (50 mg/kg/day), HFD + **11c**·**HCl** (20 mg/kg/day), HFD + **11c**·**HCl** (50 mg/kg/day), and HFD + Met (150 mg/kg/day) in 1% tween 80 were orally administered per day for 4 weeks. After the experimental period was completed, all mice were anesthetized and samples were collected. Meanwhile, body, liver, and epididymal white adipose tissues weight of mice were recorded. Serum levels of glucose, insulin, AST, ALT, TG, LDL, HDL and FFA were measured using a multifunctional biochemistry analyzer Olympus AU2700 (Olympus, Tokyo, Japan). The liver content of TG was analyzed using a diagnostic kit (Jiancheng, Nanjing, China) according to the manufacturer's instruction. The percentages were calculated using $[(V_{HFD} - V_{treatment})/V_{HFD}] \times 100\%$.

4.3.3. Glucose and insulin tolerance tests

Oral glucose tolerance test (OGTT) was performed on mice fasted for 16 h. Glucose levels of blood collected from the tail vein were determined at 0, 5,15, 30, 60, 90, and 120 min after an oral glucose load of 2.0 g/kg. For the insulin tolerance test (ITT), mice fasted for 9 h were injected intraperitoneally with 0.5 U/kg of body weight and glucose levels were measured at 0, 5, 15, 30, 60, 90, and 120 min postinjection. The insulin sensitivity was also evaluated by the formula of HOMA-IR = fasting glucose (mmol/L) × fasting insulin (μU/mL)/22.5.

4.3.4. Histology evaluation

For the histological examination, liver and epididymal fat tissues were fixed in 10% buffered formalin overnight and then embedded in paraffin, cut at a thickness of 4 μm, and stained with hematoxylin-eosin (H&E). Frozen livers were sectioned into 10 μm thick sections using a microtome, and lipid deposition was detected by two ways of staining. Oil red O staining was performed using standard protocols, and together with hematoxylin staining. All cell morphology and size were analyzed.

4.3.5. Pharmacokinetics in Sprague–Dawley rats

Two groups (n = 5) of male and female Sprague–Dawley rats (200–250 g) were fasted overnight and received **11c** and **11c**·**HCl** as an intravenous dose (5 mg/kg) or by oral gavage (20 mg/kg). Blood samples (0.4 mL)were obtained from retro-orbital bleeding at

5 min, 15 min, 30 min, 1 h, 2 h, 4 h, 6 h, 8 h, 10 h, 12 h, 14 h, and 24 h post dose for the po group. At each time point, three mice were bled resulting in a composite pharmacokinetic profile. The tubes were inverted several times to ensure mixing and placed on ice. The blood samples were centrifuged to obtain the plasma fraction. The plasma samples were deproteinized with acetonitrile containing an internal standard. After centrifugation, the supernatant was diluted and centrifuged again. The compound concentrations in the supernatant were measured by a high performance liquid chromatography–tandem mass spectrometry (LC/MS/MS).

Acknowledgments

We greatly appreciate the financial support from National Key R&D Programs of China (No. 2012ZX09103101-033), and China Postdoctoral Science Foundation (2014M552373).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.09.094.

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