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FULL-LENGTH PAPER

Scaffold-based design of xanthine as highly potent inhibitors of DPP-IV for improving glucose homeostasis in DIO mice

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Abstract Diabetes mellitus, commonly characterized by hyperglycemia, is a group of metabolic diseases. Some oral anti-diabetic drugs show poor tolerability during chronic treatment, and associate with undesired side effects. Recent advances in the understanding of physiological functions of incretins and their degrading enzyme dipeptidyl peptidase DPP-IV have led to the discovery of DPP-IV inhibitors as a new class of oral anti-diabetic drugs. Several DPP-IV inhibitors have different chemical structures of which the xanthine scaffold has specific advantages. Combining previous work with the research strategy of pharmacophore hybridization, we retained this scaffold and synthesized a new series of amino-alcohol or diamino-modified xanthine compounds. Some xanthines exhibited submicromolar inhibitory activities against DPP-IV. The most potent compound **40** (IC_{50} = 19.6 nM) exhibits a good in vivo efficacy in reducing glucose excursion at a single dose and a better chronic effect in reducing body weight than metformin in DIO mice. In other words, the combined effect improved the pathological state of DIO mice.

Keywords Xanthine scaffold · DPP-IV inhibitor · Glucose homeostasis · Pharmacophore hybridization

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Abbreviations

DPP	dipeptidyl peptidase
DIO	diet-induced obesity
HFD	high-fat diet
AUC	area under the curve
AMC	Gly-Pro-Aminomethylcoumarin
Met	metformin.

Introduction

Diabetes mellitus, commonly characterized by hyperglycemia, is a group of metabolic diseases [1]. Type 2 diabetes (T2D) accounts for approximately 90 % of all cases of diabetes, and results from an altered insulin sensitivity and impaired insulin secretion [2]. Some oral anti-diabetic drugs show poor tolerability during chronic treatment, and associate with undesired side effects, such as hypoglycemia, edema, and weight gain [3]. Recent advances in the understanding of physiological functions of incretins and their degrading enzyme dipeptidyl peptidase DPP-IV have led to the discovery of a new class of oral anti-diabetic drugs [4].

DPP-IV inhibitors possess several advantages over traditional anti-diabetic drugs, such as not leading to increased bodyweight or causing hypoglycemia, and are generally well-tolerated [5]. They were effective as monotherapy and as add-on therapy in combination with metformin, thiazolidinediones, and insulin in recent years to improve postprandial glycemic control in type 2 diabetes [6]. So far, seven small-molecule DPP-IV inhibitors have been approved by various regulatory agencies: Sitagliptin [7], Vildagliptin [8,9], Saxagliptin [10], Alogliptin [11], Linagliptin [12], Gemigliptin [13], and Teneligliptin [14] (Fig. 1).

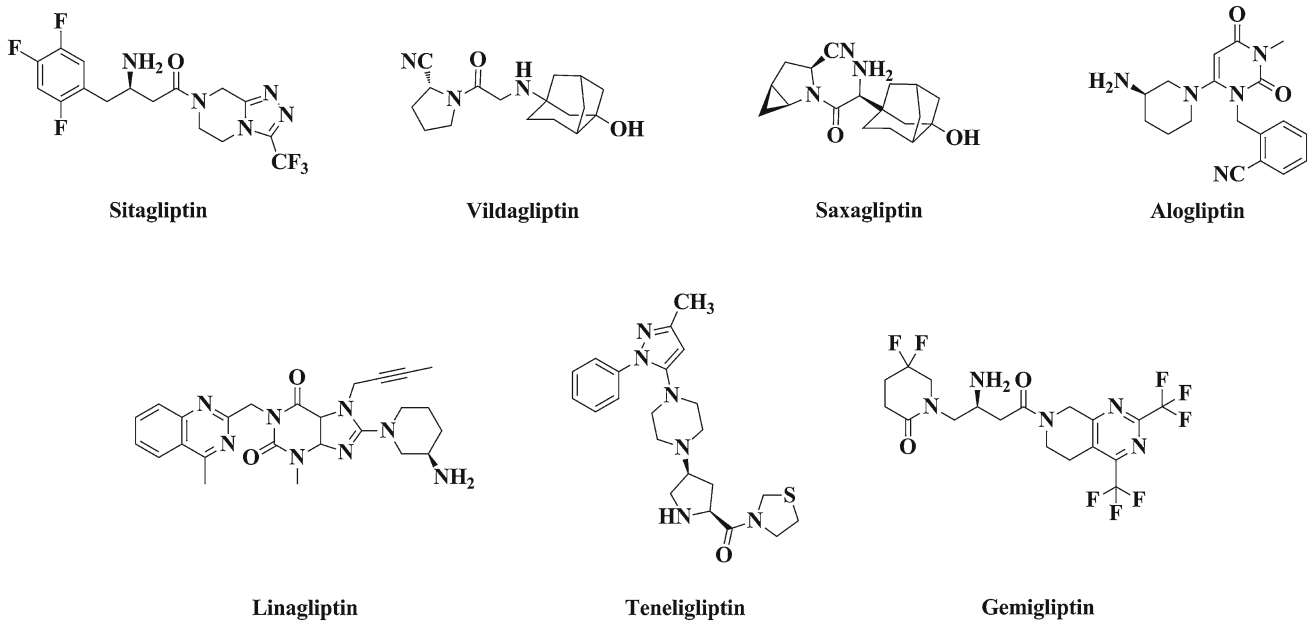


Fig. 1 DPP-IV inhibitors in the market

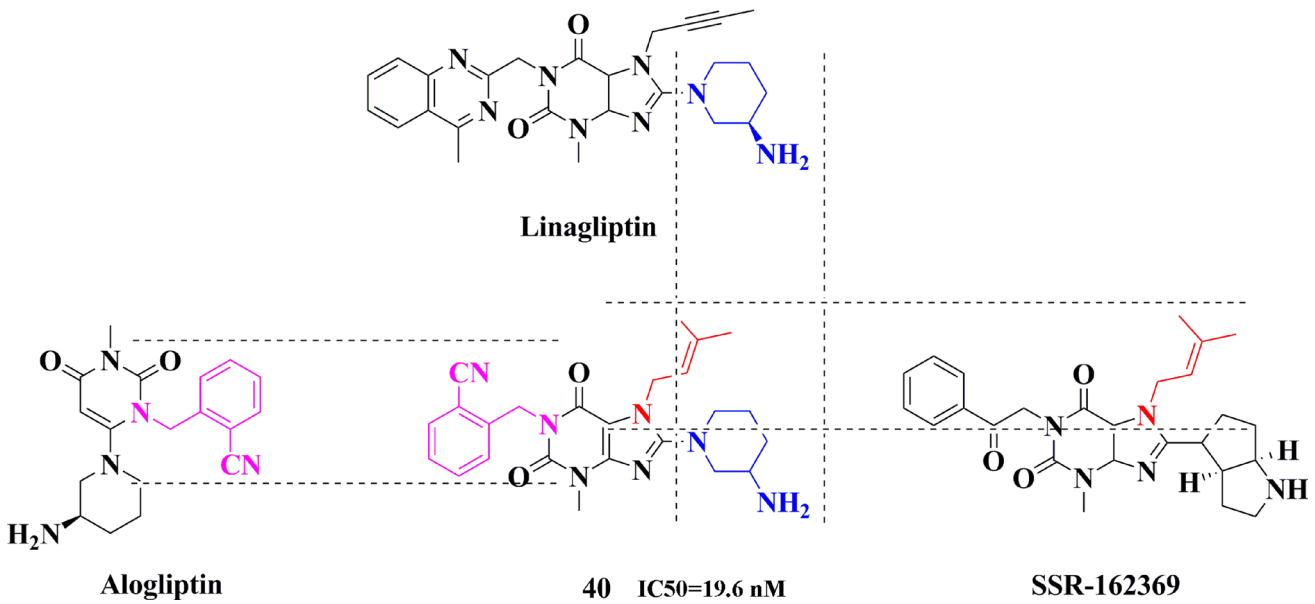
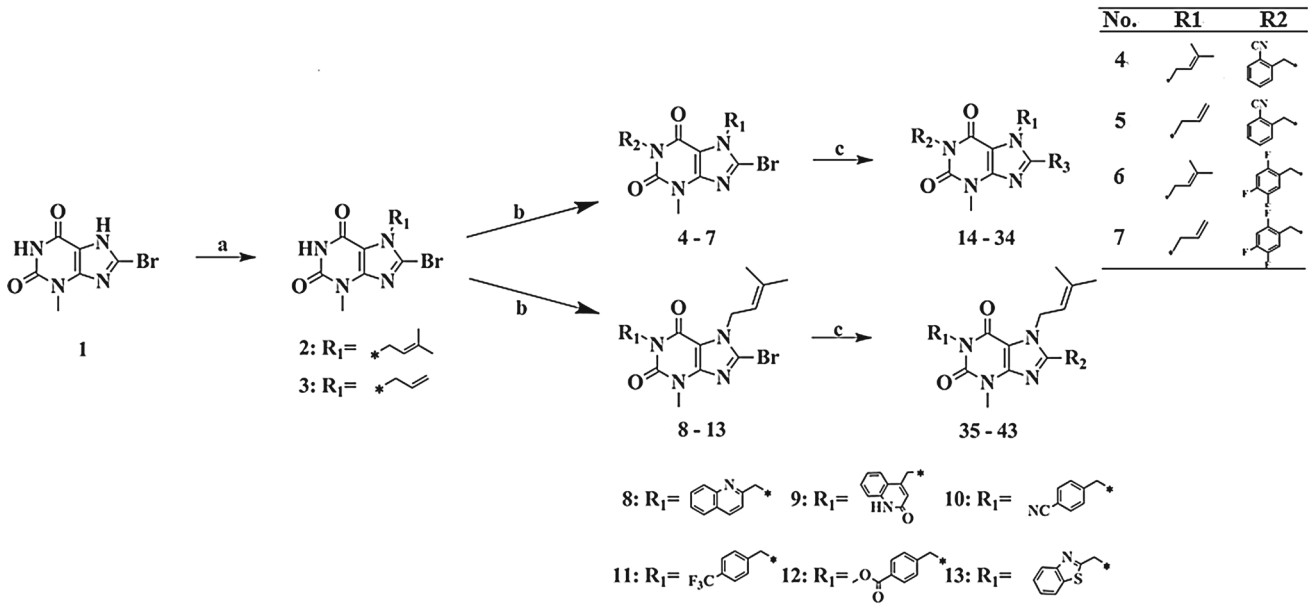


Fig. 2 Our pharmacophore hybridization strategy

Several DPP-IV inhibitors show marked structural heterogeneity (Fig. 1). Among these drugs, Linagliptin has superior potency and longer duration of action in comparison with the other marketed drugs. In addition, Linagliptin has a unique xanthine-based structure, and the distinctions between structures may account for variations in the pharmacokinetic profile. For example, Linagliptin has a long terminal half-life (up to 184 h), whereas the terminal half-life of Sitagliptin is between 10 and 12 h [15,16]. Based on the xanthine scaffold, a series of amino-alcohol or diamino- modified xanthine derivatives were designed and synthesized by combing the existing strategy of pharmacophore hybridization [17–19] (Fig. 2). We focused on finding another amino-alcohol or diamino group that can play the same role of the 3-amino piperidine group in this class of compounds, and we hoped to find a new compound having good activity in controlling blood glucose like Linagliptin. Through unremitting efforts, we obtained compound **40** (IC_{50} = 19.6 nM), which not only possessed the ability to robustly reduce glucose excursion, but also showed better chronic effect in reducing body



Scheme 1 Synthesis of **14–43**

weight than metformin. In other words, the combined effect improved the pathological state of DIO mice.

Results and discussion

Synthesis

The synthesis routes of compounds **14–34** and **35–43** are outlined in Scheme 1. Generally, the alkylation of commercially available material **1** with 1-bromo-3-methylbut-2-ene or 3-bromoprop-1-ene offered intermediate **2** or **3**. Compounds **4–13** were obtained by the N-alkylation of compounds **2** or **3**. The targeted compounds **14–34** and **35–43** were obtained by the replacement of the bromine group with an amino-alcohol or diamino group. For the synthesis of these targeted compounds, conventional heating and microwave irradiation were employed based on respective advantages.

Amino-alcohol or diamino-modified xanthines

Twenty-one novel amino-alcohol or diamino-modified xanthines compounds were synthesized and evaluated for their inhibition of DPP-IV in vitro (Table 1). Unfortunately, most of these compounds did not possess the anticipated inhibitory activity except for some ones. In general, changing the 2-cyanobenzyl group to 2,4,5-trifluorobenzyl in N-R_1 decreased by half in potency (**14**: 96.8 % vs. **15**: 37.0 %; **16**: 95.9 % vs. **17**: 41.9 %; **18**: 47.2 % vs. **19**: 22.1 %). 3-Methyl-2-butenyl-substituted xanthines in N-R_2 showed less than 20 % increase in potency than allyl-substituted (**19**: 22.1 % vs. **20**: 9.2 %). However, among these compounds, the replacement in N-R_3 led to bigger changes in activity than in N-R_1 and N-R_2. For instance, changing the piperidyl group to 4-(hydroxymethyl)piperidin-1-yl caused a 5-fold decrease (**17**: 41.9 %, **27**: 8.2 %) to a 95-fold (**16**: 95.9 %, **26**: 1.3 %) decrease in activity, respectively. Above all, encouraged by the more than 95 % inhibition of some compounds (**14**: 96.8 %, **16**: 95.9 %), compound **14** was selected for further optimization toward the discovery of highly potent DPP-IV inhibitors.

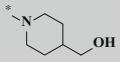
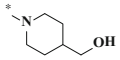
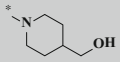
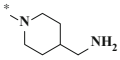
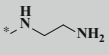
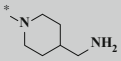
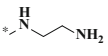
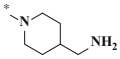
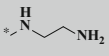
Optimization of compound **14**

Five analogous (**35–39**) compounds were synthesized, whose substituted groups were different from compounds **14** on the N-R_2. Besides, we also introduced the 3-aminopiperidinyl group (**40–43**) on the N-R_3 (Table 2). In general, piperidyl containing analogs showed poorer inhibition rates than the 3-aminopiperidinyl counterparts. Further activity determination in vitro showed that, as depicted in Table 2, compounds **40** and **43** exhibited potent IC_{50} values in the low nanomolar range (**40**: IC_{50} = 19.6 nM; **43**: IC_{50} = 13.4 nM) against DPP-IV and exhibited excellent selectivity against DPP-8 and DPP-9.

In vivo biological evaluations

Compounds **14**, **35**, **40**, **41**, and **43** in Table 2 were chosen for in vivo evaluation. In the oral glucose tolerance test (OGTT), these compounds were administered orally at a single dose of 5 mg/kg. The result shows that their reducing glucose

Table 1 DPP-IV inhibitory profiles of compounds **14–34**

No.	R_1	R_2	R_3	Percent inhibition (at 5 μM)	No.	R_1	R_2	R_3	Percent inhibition (at 5 μM)
14				96.8	**25**				7.5
15				37.0	**26**				1.3
16				95.9	**27**				8.2
17				41.9	**28**				4.2
18				47.2	**29**				50.9
19				22.1	**30**				-14.7
20				9.2	**31**				-23.3
21				-13.4	**32**				8.3
22				-16.1	**33**				-21.5
23				42.9	**34**				-24.2
24				-10.9					

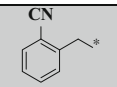
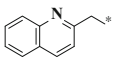
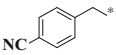
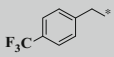
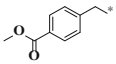
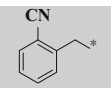
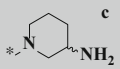
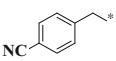
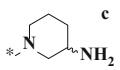
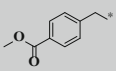
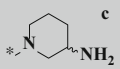
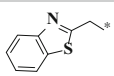
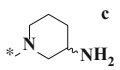
Twenty-one compounds were tested at a concentration of 5 μM. The percent inhibition is an average of three independent titrations, having calculated standard errors below 15 %

excursion effects are superior to metformin (150 mg/kg) (Fig. 3). In contrast, compounds **40** and **43** exhibited significantly reduced glucose excursion (39.1 and 49.0 %, respectively) at a single dose in DIO mice. Considering the good hypoglycemic activities, compounds **40** and **43** were selected for further studies in improving the pathological state of DIO mice. As illustrated in Fig. 4, compound **40** was better than metformin to reduce body weight of DIO mice at a dosage of 5 mg/kg/day and 150 mg/kg/day, respectively. On the other hand, compound **40** effectively lowered the liver and fat weight of DIO mice (11.47 and 34.78 %, respectively). On the basis of its DPP-IV potency, selectivity, in vivo efficacy, compound **40** was chosen for further development.

Conclusions

In summary, we have developed a new series of amino-alcohol or diamino-modified xanthine compounds as DPP-IV inhibitors. In applying our strategy of pharmacophore hybridization, we successfully generated highly potent DPP-IV inhibitor **40** (IC_{50} = 19.6 nM) with good selectivity against DPP-8 and DPP-9. In addition, compound **40** exhibited better chronic effect in reducing body weight than metformin, while possessing the ability to robustly reduce glucose excursion. Overall, compound **40** effectively improved the pathological state of DIO mice, was chosen for further development.

Table 2 DPP inhibitory profiles of compounds **14**, and **35–43**

No.	R_1	R_2	Percent inhibition (at 5 μM)	IC_{50}		
				DPP-8 (μM)	DPP-9 (μM)	DPP-IV (nM)
14			96.8	> 100	> 100	265±12.4
35			102.4	> 100	> 100	157±7.3
36			85.1	NT[b]	NT	NT
37			74.2	NT	NT	NT
38			55.3	NT	NT	NT
39			74.6	NT	NT	NT
40			99.8	> 100	> 100	19.6±1.4
41			95.5	> 100	> 100	140±8.4
42			96.3	NT	NT	NT
43			98.8	> 100	> 100	13.4±2.0

Nine compounds were tested at a concentration of 5 μM. The percent inhibition and IC_{50} results are an average of three independent titrations, having calculated standard errors below 15 %.

[b] NT = not tested

[c] Compounds (40–43) are racemates

Materials and methods

General

Chemistry reagents of analytical grade were purchased from Changzheng Chemical Factory (Chengdu, Sichuan, PR China) and were used without further purification. TLC was performed on 0.20 mm Silica Gel 60 F_{254} plates (Qingdao Ocean Chemical Factory, Shandong, China). ^1H and ^{13}C NMR spectra were recorded at 400 MHz and 100 MHz, respectively, 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; 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 (Micromass, Manchester, UK). All the microwave irradiation experiments were performed in a CEM system, and reaction temperatures were monitored by an equipped IR sensor (Discover and Explorer SP, CEM).

8-Bromo-3-methyl-7-(3-methylbut-2-enyl)-1H-purine-2,6(3H, 7H)-dione (**2**)

1-Bromo-3-methylbut-2-ene (1 mmol) was added into a solution of 8-bromo-3-methyl-1H-purine-2,6(3H, 7H)-dione (1 mmol) and DIEA (1 mmol) in DMF (2 mL). The resulting solution was heated to 80 °C and stirred at this temperature for 4 h. After cooling to ambient temperature, ice-cold water (10 mL) was added. The precipitate was separated by filtra-

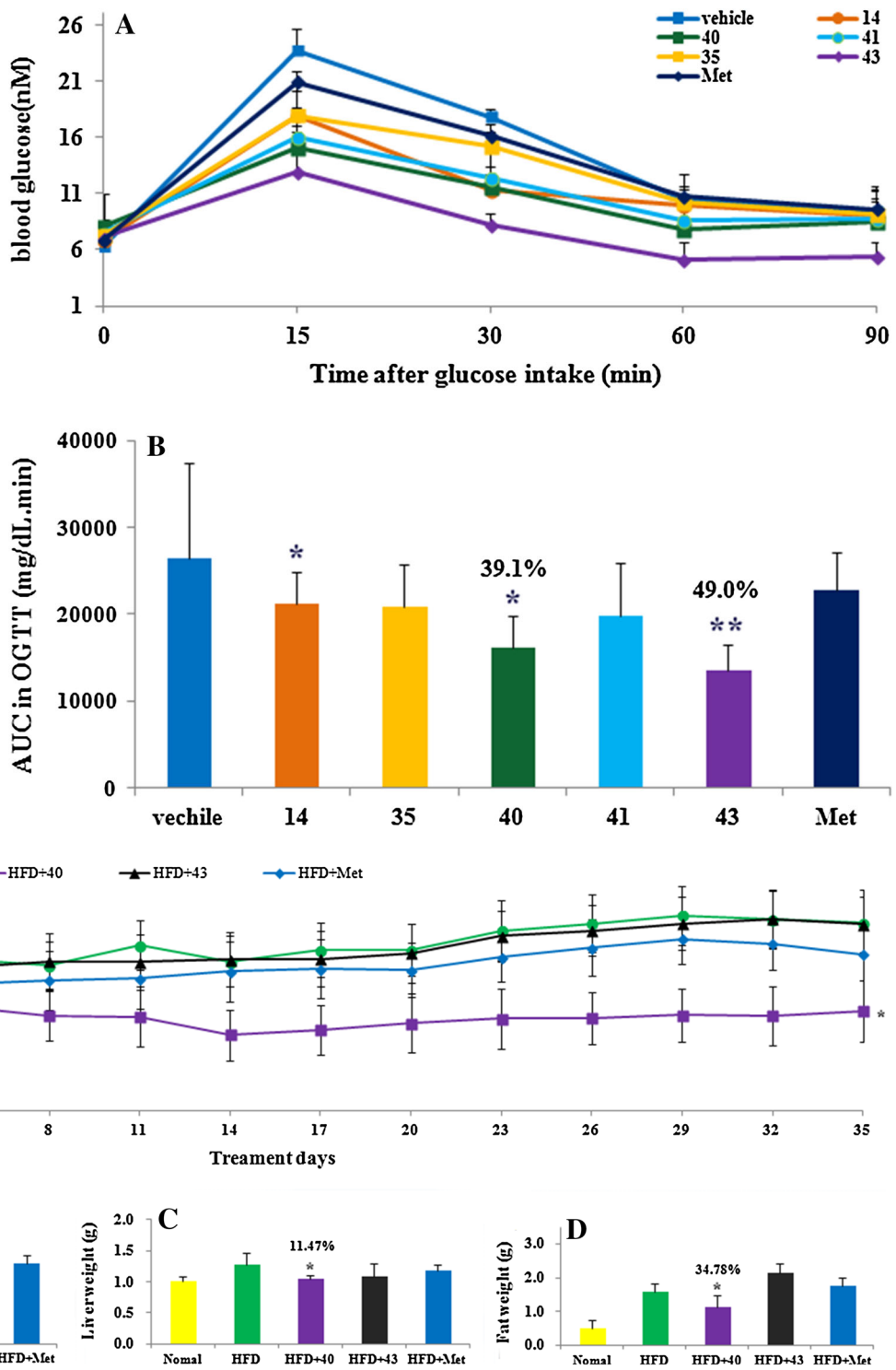


Fig. 3 Effect of compounds in the oral glucose tolerance test at a single dose. Change of blood glucose level **a** and AUC value of blood glucose between 0 and 90 min **b** in an oral glucose tolerance test in male C57BL/6J mice fed a high-fat diet. The selected compounds (5 mg/kg), Metformin (150 mg/kg), or water (vehicle) was administered 30 min prior to an oral glucose challenge (2.0 g/kg). Data are mean ± SEM ($n = 5$/group). $p \leq 0.05$; ** $p \leq 0.01$ versus vehicle

Fig. 4 Chronic efficacy studies of compounds **40** and **43** in DIO mice: **a** daily body weight; either HFD group or treated groups were monitored during the oral administration of metformin (150 mg/kg/day), **40** (5 mg/kg/day) or **43** (5 mg/kg/day) for 5 weeks; **b** cumulative food intake; **c** liver weight; **d** fat weight. $*p \leq 0.05$ versus the corresponding HFD group

tion, washed with water and a small portion of diethylether, and then dried to give the product **2**.White solid; Yield 85.3 %; mp 227–229 °C. ^{1}H NMR (400 MHz, DMSO-d$_6$): δ 11.27 (s, 1H), 5.24 (t, 1H, $J = 6.8$ Hz), 4.86 (d, 2H, $J = 6.8$ Hz), 3.31 (s, 3H), 1.80 (s, 3H), 1.70 (s, 3H); MS (ESI), m/z: 313.06, 135.05 $[M+H]^+$.

7-Allyl-8-bromo-3-methyl-1H-purine-2,6(3H,7H)-dione (**3**)

The title compound was prepared from 3-bromoprop-1-ene according to the procedure, for example **2**. Light yellow solid; Yield 82.1 %; mp 231–233 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.97 (s, 1H), 4.33 (t, 2H, $J = 6.0$ Hz), 4.01 (t, 2H, $J = 6.0$ Hz), 3.31 (s, 3H), 3.20-3.10 (m, 4H), 1.69–1.60 (m, 4H), 1.60–1.51 (m, 2H); MS (ESI), m/z: 285.38, 287.35 $[M+H]^+$.

2-((8-Bromo-3-methyl-7-(3-methylbut-2-enyl)-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-1-yl) methyl)benzonitrile (**4**)

2-(Bromomethyl)benzonitrile (1.1 mmol) was added to a suspension of **2** (1 mmol) and K_2CO_3 (1.6 mmol) in DMF (2 mL). The mixture was stirred at ambient temperature for 6 h. Then, water was added, and the resulting precipitate was separated by filtration, and washed with water to obtain desired compound **4**. White solid; Yield 82.2 %; mp 135–137 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ; 7.85 (d, 1H, $J = 7.2$ Hz), 7.63 (d, 1H, $J = 7.6$ Hz), 7.47 (d, 1H, $J = 7.6$ Hz), 7.31 (d, 1H, $J = 7.2$ Hz), 5.27–5.24 (m, 1H), 5.23 (s, 2H), 4.92 (d, 2H, $J = 5.2$ Hz), 3.40 (s, 3H), 1.80 (s, 3H), 1.69 (s, 3H); MS (ESI), m/z: 466.40, 468.38 $[M+K]^+$.

2-((7-Allyl-8-bromo-3-methyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-1-yl) methyl)benzonitrile (**5**)

2-(Bromomethyl)benzonitrile (1.1 mmol) was added to a suspension of **3** (1 mmol) and K_2CO_3 (1.6 mmol) in DMF (2 mL). The mixture was stirred at ambient temperature for 6 h. Then, water was added, and the resulting precipitate was separated by filtration, and washed with water to obtain desired compound **5**. White solid; Yield 63.6 %; mp 127–129 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.84 (d, 1H, $J = 7.6$ Hz), 7.63 (t, 1H, $J = 7.6$ Hz), 7.47 (t, 1H, $J = 7.6$ Hz), 7.30 (d, 1H, $J = 7.6$ Hz), 6.04–5.92 (m, 1H), 5.24 (d, 1H, $J = 16.8$ Hz), 5.22 (s, 2H), 5.02 (d, 1H, $J = 16.8$ Hz), 4.92 (s, 2H), 3.42 (s, 3H); MS (ESI), m/z: 438.09, 440.09 $[M+K]^+$.

8-Bromo-3-methyl-7-(3-methylbut-2-enyl)-1-(2,4,5-trifluorobenzyl)-1H-purine-2,6(3H,7H)-dione (**6**)

The title compound was prepared from 1-(bromomethyl)-2,4,5-trifluorobenzene according to the procedure, for example **4**. White solid; Yield 68.5 %; mp 147–149 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.60–7.49 (m, 1H), 7.37–7.28 (m, 1H), 5.26 (t, 1H, $J = 7.2$ Hz), 5.04 (s, 2H), 4.92 (d, 2H, $J = 7.2$ Hz), 3.39 (s, 3H), 1.80 (s, 3H), 1.69 (s, 3H); MS (ESI), m/z: 495.11, 497.07 $[M+K]^+$.

7-Allyl-8-bromo-3-methyl-1-(2,4,5-trifluorobenzyl)-1H-purine-2,6(3H,7H)-dione (**7**)

The title compound was prepared from 1-(bromomethyl)-2,4,5-trifluorobenzene according to the procedure, for example **5**. White solid; Yield 63.4 %; mp 116–118 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.59–7.49 (m, 1H), 7.36–7.26 (m, 1H), 6.04–5.90 (m, 1H), 5.24 (d, 1H, $J = 10.4$ Hz), 5.03 (s, 2H), 5.01 (d, 1H, $J = 10.4$ Hz), 4.92 (d, 2H, $J = 4.8$ Hz), 3.41 (s, 3H); MS (ESI), m/z: 467.09, 469.08 $[M+K]^+$.

8-Bromo-3-methyl-7-(3-methylbut-2-enyl)-1-(quinolin-2-ylmethyl)-1H-purine- 2,6(3H,7H)-dione (**8**)

The title compound was prepared from 2-(chloromethyl) quinoline hydrochloride according to the procedure, for example **4**. White solid; Yield 62.5 %; mp 207–209 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.10 (d, 1H, $J = 8.0$ Hz), 8.00 (d, 1H, $J = 8.8$ Hz), 7.77 (d, 1H, $J = 8.0$ Hz), 7.66 (t, 1H, $J = 7.6$ Hz), 7.49 (t, 1H, $J = 7.6$ Hz), 7.33 (d, 1H, $J = 8.8$ Hz), 5.51 (s, 2H), 5.32 (t, 1H, $J = 6.8$ Hz), 4.99 (d, 2H, $J = 6.8$ Hz), 3.58 (s, 3H), 1.83 (s, 3H), 1.73 (s, 3H); MS (ESI), m/z: 454.03, 456.02 $[M+H]^+$.

8-Bromo-3-methyl-7-(3-methylbut-2-enyl)-1-((2-oxo-1,2-dihydroquinolin-4-yl)methyl)-1H-purine-2,6(3H,7H)-dione (**9**)

The title compound was prepared from 4-(bromomethyl) quinolin-2(1H)-one according to the procedure for example **4**. White solid; Yield 60.5 %; mp 290–292 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.69 (s, 1H), 7.92 (d, 1H, $J = 7.6$ Hz), 7.56 (t, 1H, $J = 7.6$ Hz), 7.37 (d, 1H, $J = 7.6$ Hz), 7.27 (t, 1H, $J = 7.6$ Hz), 6.01 (s, 1H), 5.28 (s, 2H), 4.92 (d, 2H, $J = 6.4$ Hz), 3.44 (s, 3H), 1.79 (s, 3H), 1.69 (s, 3H); MS (ESI), m/z: 508.18, 510.18 $[M+K]^+$.

4-((8-Bromo-3-methyl-7-(3-methylbut-2-enyl)-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-1-yl)methyl) benzonitrile (**10**)

The title compound was prepared from 4-(chloromethyl) benzonitrile according to the procedure, for example **4**. White solid; mp 236–238 °C. Yield 64.6 %; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.61 (q, 4H, $J = 7.6$ Hz), 5.29 (t, 1H, $J = 6.8$ Hz), 5.21 (s, 2H), 4.97 (d, 2H, $J = 6.8$ Hz), 3.54 (s, 3H), 1.86 (s, 3H), 1.74 (s, 3H); MS (ESI), m/z: 466.02, 468.01 $[M+K]^+$.

8-Bromo-3-methyl-7-(3-methylbut-2-enyl)-1-(4-(trifluoromethyl)benzyl)-1H-purine-2,6(3H,7H)-dione (**11**)

The title compound was prepared from 1-(chloromethyl)-4-(trifluoromethyl) -benzene according to the procedure, for example **4**. White solid; Yield 63.7 %; mp 189–191 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.61–7.53 (m, 4H), 5.30 (t, 1H, J = 6.8 Hz), 5.23 (s, 2H), 4.48 (d, 2H, J = 6.8 Hz), 3.54 (s, 3H), 1.86 (s, 3H), 1.74 (s, 3H); MS (ESI), m/z: 508.94, 510.93 $[M+K]^+$.

Methyl-4-((8-bromo-3-methyl-7-(3-methylbut-2-enyl)-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-1-yl)methyl)benzoate (**12**)

The title compound was prepared from methyl 4-(chloromethyl)benzoate according to the procedure, for example **4**. White solid; Yield 68.2 %; mp 217–219 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.98 (d, 2H, J = 8.0 Hz), 7.52 (d, 2H, J = 8.0 Hz), 5.30 (t, 1H, J = 6.8 Hz), 5.23 (s, 2H), 4.98 (d, 2H, J = 6.8 Hz), 3.89 (s, 3H), 3.54 (s, 3H), 1.86 (s, 3H), 1.74 (s, 3H); MS (ESI), m/z: 499.20, 501.15 $[M+K]^+$.

1-(Benzo[d]thiazol-2-ylmethyl)-8-bromo-3-methyl-7-(3-methylbut-2-enyl)-1H-purine-2,6(3H,7H)-dione (**13**)

The title compound was prepared from 2-(chloromethyl) benzo[d]thiazole according to the procedure for example **4**. Yellow solid; Yield 69.2 %; mp 249–251 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.06 (d, 1H, J = 8.0 Hz), 7.95 (d, 1H, J = 8.0 Hz), 7.51 (t, 1H, J = 7.6 Hz), 7.44 (t, 1H, J = 7.2 Hz), 5.48 (s, 2H), 5.25 (t, 1H, J = 6.4 Hz), 4.93 (d, 2H, J = 6.4 Hz), 3.43 (s, 3H), 1.80 (s, 3H), 1.69 (s, 3H); MS (ESI), m/z: 498.14, 500.14 $[M+K]^+$.

2-((3-Methyl-7-(3-methylbut-2-enyl)-2,6-dioxo-8-(piperazine-1-yl)-2,3,6,7-tetrahydro-1H-purin-1-yl)methyl)benzonitrile (**14**)

A mixture of **4** (1 mmol), K_2CO_3 (2 mmol), and piperazine (1.1 mmol) in DMF (2 mL) was introduced into a CEM Discover microwave reaction vessel equipped with a magnetic stirrer. The vessel was sealed, and the reaction mixture was stirred for 5 min at rt and continuously irradiated at 30 W for 25 min at 140 °C twice. The resulting mixture was cooled to room temperature. Then, cold water was added, and the resulting precipitate was separated by filtration to afford the target compound **14**. White solid; Yield 72.5 %; mp 179–181 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.84 (d, 1H, J = 7.6 Hz), 7.63 (t, 1H, J = 7.6 Hz), 7.46 (t, 1H, J = 7.6 Hz), 7.25 (d, 1H, J = 7.6 Hz), 5.35 (t, 1H, J = 6.4 Hz), 5.21 (s, 2H), 4.68 (d, 2H, J = 6.4 Hz), 3.39 (s, 3H), 3.17–3.09 (m, 4H), 2.86–2.79 (m, 4H), 2.41–2.30 (m, 1H), 1.70 (s, 3H), 1.68 (s, 3H); MS (ESI), m/z: 434.65 $[M + H]^+$; HPLC purity = 99.1 %.

3-Methyl-7-(3-methylbut-2-enyl)-8-(piperazin-1-yl)-1-(2,4,5-trifluorobenzyl)-1H-purine-2,6(3H,7H)-dione (**15**)

The title compound was prepared from 8-bromo-3-methyl-7-(3-methylbut-2-enyl)-1-(2,4,5-trifluorobenzyl)-1H-purine-2,6(3H,7H)-dione (**6**) according to the procedure, for example **14**. White solid; Yield 78.3 %; mp 144–146 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.59–7.48 (m, 1H), 7.30–7.18 (m, 1H), 5.34 (t, 1H, J = 6.0 Hz), 5.03 (s, 2H), 4.67 (d, 2H, J = 6.0 Hz), 3.38 (s, 3H), 3.16–3.08 (m, 4H), 2.85–2.77 (m, 4H), 2.40–2.29 (m, 1H), 1.70 (s, 3H), 1.67 (s, 3H); MS (ESI), m/z: 463.32 $[M+H]^+$; HPLC purity = 96.7 %.

2-((7-Allyl-3-methyl-2,6-dioxo-8-(piperazin-1-yl)-2,3,6,7-tetrahydro-1H-purin-1-yl)methyl)benzonitrile (**16**)

The title compound was prepared from 2-((7-allyl-8-bromo-3-methyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-1-yl)methyl)benzonitrile (**5**) according to the procedure, for example **14**. White solid; Yield 82.7 %; mp 156–158 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.83 (d, 1H, J = 7.6 Hz), 7.62 (t, 1H, J = 7.6 Hz), 7.46 (t, 1H, J = 7.6 Hz), 7.24 (d, 1H, J = 7.6 Hz), 6.09–5.95 (m, 1H), 5.21 (d, 1H, J = 9.2 Hz), 5.20 (s, 2H), 5.10 (d, 1H, J = 17.2 Hz), 4.71 (d, 2H, J= 4.4 Hz), 3.40 (s, 3H), 3.22–3.13 (m, 4H), 2.86–2.78 (m, 4H); MS (ESI), m/z: 406.94 $[M + H]^+$; HPLC purity = 98.7 %.

7-Allyl-3-methyl-8-(piperazin-1-yl)-1-(2,4,5-trifluorobenzyl)-1H-purine-2,6(3H,7H)-dione (**17**)

The title compound was prepared from 7-allyl-8-bromo-3-methyl-1-(2,4,5-trifluorobenzyl)-1H-purine-2,6(3H,7H)-dione (**7**) according to the procedure, for example **14**. White solid; Yield 87.2 %; mp 161–163 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.59-7.47 (m, 1H), 7.29–7.18 (m, 1H), 6.07–5.95 (m, 1H), 5.21 (d, 1H, J = 10.4 Hz), 5.09 (d, 1H, J = 17.2 Hz), 5.02 (s, 2H), 4.70 (d, 2H, J = 4.0 Hz), 3.39 (s, 3H), 3.20–3.10 (m, 4H), 2.85–2.76 (m, 4H), 2.45–2.33 (m, 1H); MS (ESI), m/z: 434.91 $[M+H]^+$; HPLC purity = 99.3 %.

2-((8-(2-Aminoethylamino)-3-methyl-7-(3-methylbut-2-enyl)-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-1-yl)methyl)benzonitrile (**18**)

A mixture of **4** (1 mmol), K_2CO_3 (2 mmol), and ethane-1,2-diamine (1.1 mmol) in DMF (2 mL) was introduced into a CEM Discover microwave reaction vessel equipped with a magnetic stirrer. The vessel was sealed, and the reaction mixture was stirred for 5 min at rt and continuously irradiated at 30 W for 30 min at 140 °C twice. The resulting mixture was cooled to room temperature. Then, cold water was added, and the resulting precipitate was separated by filtration to afford target compound **18**. White solid; Yield 64.3 %; mp 160–162 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.83 (d, 1H, J = 7.6 Hz), 7.60 (t, 1H, J = 7.6 Hz), 7.43 (t, 1H, J = 7.6 Hz), 7.20 (d, 1H, J = 7.6 Hz), 5.21 (t, 1H, J = 6.4 Hz), 5.18 (s, 2H), 4.68 (d, 2H, J = 6.4 Hz), 3.36 (s, 3H), 3.34 (d, 2H, J = 6.0 Hz), 2.75 (t, 2H, J = 6.0 Hz), 1.72 (s, 3H), 1.67 (s, 3H); MS (ESI), m/z: 408.08 $[M+H]^+$; HPLC purity = 98.1 %.

8-(2-Aminoethylamino)-3-methyl-7-(3-methylbut-2-enyl)-1-(2,4,5-trifluorobenzyl)-1H-purine-2,6(3H,7H)-dione (**19**)

The title compound was prepared from 8-bromo-3-methyl-7-(3-methylbut-2-enyl)-1-(2,4,5-trifluorobenzyl)-1H-purine-2,6(3H,7H)-dione (**6**) according to the procedure, for example **18**. White solid; Yield 51.5 %; mp 161–163 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.59–7.47 (m, 1H), 7.24–7.15 (m, 1H), 6.11–5.86 (m, 1H), 5.20 (t, 1H, J = 6.0 Hz), 5.00 (s, 2H), 4.67 (d, 2H, J = 6.0 Hz), 3.35 (s, 3H), 3.32 (d, 2H, J = 6.4 Hz), 2.74 (t, 2H, J = 6.4 Hz), 1.72 (s, 3H), 1.65 (s, 3H), 1.59–1.42 (m, 2H); MS (ESI), m/z: 437.09 $[M + H]^+$; HPLC purity = 97.8 %.

7-Allyl-8-(2-aminoethylamino)-3-methyl-1-(2,4,5-trifluorobenzyl)-1H-purine-2,6(3H,7H)-dione (**20**)

The title compound was prepared from 7-allyl-8-bromo-3-methyl-1-(2,4,5-trifluorobenzyl)-1H-purine-2,6(3H,7H)-dione (**7**) according to the procedure, for example **18**. White solid; Yield 63.6 %; mp 186–188 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.59–7.47 (m, 1H), 7.25–7.13 (m, 1H), 5.97–5.80 (m, 1H), 5.14 (d, 1H, J = 10.4 Hz), 5.00 (s, 2H), 4.99 (d, 1H, J = 17.6 Hz), 4.69 (d, 2H, J = 4.4 Hz), 3.37 (s, 3H), 3.32 (d, 2H, J = 6.4 Hz), 2.73 (t, 2H, J = 6.4 Hz), 1.67–1.17 (m, 2H); MS (ESI), m/z: 407.19 $[M + H]^-$; HPLC purity = 97.7 %.

2-((8-(4-(2-Hydroxyethyl)piperazin-1-yl)-3-methyl-7-(3-methylbut-2-enyl)-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-1-yl)methyl)benzonitrile (**21**)

A mixture of **4** (1 mmol), K_2CO_3 (2 mmol), and 2-(piperazin-1-yl)ethanol (1.1 mmol) in DMF (2 mL) was introduced into a CEM Discover microwave reaction vessel equipped with a magnetic stirrer. The vessel was sealed, and the reaction mixture was stirred for 5 min at rt and continuously irradiated at 30 W for 25 min at 140 °C twice. The resulting mixture was cooled to room temperature. Then, cold water was added, and the resulting precipitate was separated by filtration to afford target compound **21**. White solid; Yield 76.1 %; mp 182–184 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.84 (d, 1H, J = 8.0 Hz), 7.63 (t, 1H, J = 7.6 Hz), 7.46 (t, 1H, J = 7.6 Hz), 7.25 (d, 1H, J = 8.0 Hz), 5.33 (t, 1H, J = 6.0 Hz), 5.21 (s, 2H), 4.69 (d, 2H, J = 6.0 Hz), 4.44 (t, 1H, J = 5.2 Hz), 3.54 (q, 2H, J = 6.0 Hz), 3.39 (s, 3H), 3.27–3.19 (m, 4H), 2.64–2.54 (m, 4H), 2.45 (t, 2H, J = 6.0 Hz), 1.70 (s, 3H), 1.68 (s, 3H); MS (ESI), m/z: 478.66 $[M+H]^+$; HPLC purity = 98.3 %.

8-(4-(2-Hydroxyethyl)piperazin-1-yl)-3-methyl-7-(3-methylbut-2-enyl)-1-(2,4,5-trifluorobenzyl)-1H-purine-2,6(3H,7H)-dione (**22**)

The title compound was prepared from 8-bromo-3-methyl-7-(3-methylbut-2-enyl)-1-(2,4,5-trifluorobenzyl)-1H-purine-2,6(3H,7H)-dione (**6**) according to the procedure, for example **21**. White solid; Yield 71.8 %; mp 123–125 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.61–7.47 (m, 1H), 7.30–7.18 (m, 1H), 5.32 (t, 1H, J = 6.0 Hz), 5.03 (s, 2H), 4.68 (d, 2H, J = 6.0 Hz), 4.44 (t, 1H, J = 5.2 Hz), 3.55 (q, 2H, J = 6.0 Hz), 3.38 (s, 3H), 3.25–3.16 (m, 4H), 2.61–2.54 (m, 4H), 2.46 (t, 2H, J = 6.0 Hz), 1.70 (s, 3H), 1.67 (s, 3H); MS (ESI), m/z: 507.59 $[M+H]^+$; HPLC purity = 98.6 %.

2-((7-Allyl-8-(4-(2-hydroxyethyl)piperazin-1-yl)-3-methyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-1-yl)methyl)benzonitrile (**23**)

The title compound was prepared from 2-((7-allyl-8-bromo-3-methyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-1-yl)methyl)benzonitrile (**5**) according to the procedure, for example **21**. White solid; Yield 75.7 %; mp 137–139 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.83 (d, 1H, J = 7.6 Hz), 7.62 (t, 1H, J = 8.0 Hz), 7.46 (t, 1H, J = 7.6 Hz), 7.24 (d, 1H, J = 8.0 Hz), 6.08–5.95 (m, 1H), 5.21 (d, 1H, J = 9.2 Hz), 5.20 (s, 2H), 5.10 (d, 1H, J = 17.2 Hz), 4.72 (d, 2H, J = 4.0 Hz), 4.44 (t, 1H, J = 4.0 Hz), 3.55 (q, 2H,

J = 5.6 Hz), 3.40 (s, 3H), 3.29–3.20 (m, 4H), 2.62–2.53 (m, 4H), 2.45 (t, 2H, J = 5.6 Hz); MS (ESI), m/z: 450.39 $[M+H]^+$; HPLC purity = 98.3 %.

7-Allyl-8-(4-(2-hydroxyethyl)piperazin-1-yl)-3-methyl-1-(2,4,5-trifluorobenzyl)-1H-purine-2,6(3H,7H)-dione (**24**)

The title compound was prepared from 7-allyl-8-bromo-3-methyl-1-(2,4,5-trifluorobenzyl)-1H-purine-2,6(3H,7H)-dione (**7**) according to the procedure, for example **21**. White solid; Yield 72.3 %; mp 150–152 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.57–7.46 (m, 1H), 7.28–7.17 (m, 1H), 6.08–5.96 (m, 1H), 5.21 (d, 1H, J = 10.4 Hz), 5.09 (d, 1H, J = 17.2 Hz), 5.01 (s, 2H), 4.71 (d, 2H, J = 3.2 Hz), 4.49 (t, 1H, J = 3.2 Hz), 3.54 (q, 2H, J = 6.0 Hz), 3.36 (s, 3H), 3.30–3.21 (m, 4H), 2.61–2.53 (m, 4H), 2.46 (t, 2H, J = 6.0 Hz); MS (ESI), m/z: 479.75 $[M + H]^+$; HPLC purity = 98.5 %.

8-(4-(Hydroxymethyl)piperidin-1-yl)-3-methyl-7-(3-methylbut-2-enyl)-1-(2,4,5-trifluorobenzyl)-1H-purine-2,6(3H,7H)-dione (**25**)

A mixture of **4** (1 mmol), K_2CO_3 (2 mmol), and piperidin-4-ylmethanol (1.1 mmol) in DMF (2 mL) was introduced into a CEM Discover microwave reaction vessel equipped with a magnetic stirrer. The vessel was sealed, and the reaction mixture was stirred for 5 min at rt and continuously irradiated at 30 W for 25 min at 140 °C twice. The resulting mixture was cooled to room temperature. Then, cold water was added, and the resulting precipitate was separated by filtration to afford target compound **25**. White solid; Yield 70.3 %; mp 147–149 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.59–7.48 (m, 1H), 7.29–7.20 (m, 1H), 5.33 (t, 1H, J = 6.4 Hz), 5.03 (s, 2H), 4.65 (d, 2H, J = 6.4 Hz), 4.53–4.51 (m, 1H), 3.54 (d, 2H, J = 12.0 Hz), 3.37 (s, 3H), 3.32–3.27 (m, 2H), 2.93 (t, 2H, J = 12.0 Hz), 1.77–1.74 (m, 2H), 1.70 (s, 3H), 1.67 (s, 3H), 1.63–1.50 (m, 1H), 1.34–1.24 (m, 2H); MS (ESI), m/z: 492.32 $[M+H]^+$; HPLC purity = 98.4 %.

2-((7-Allyl-8-(4-(hydroxymethyl)piperidin-1-yl)-3-methyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-1-yl)methyl)benzonitrile (**26**)

The title compound was prepared from 2-((7-allyl-8-bromo-3-methyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-1-yl)methyl)benzonitrile (**5**) according to the procedure, for example **25**. White solid; Yield 70.4 %; mp 134–136 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.83 (d, 1H, J = 7.6 Hz), 7.62 (t, 1H, J = 8.0 Hz), 7.46 (t, 1H, J = 7.6 Hz), 7.24 (d, 1H, J = 8.0 Hz), 6.10–5.95 (m, 1H), 5.20 (s, 2H), 5.19 (d, 1H, J = 9.2 Hz), 5.10 (d, 1H, J = 17.2 Hz), 4.67 (d, 2H, J = 5.2 Hz), 4.52 (t, 1H, J = 5.2 Hz), 3.61 (d, 2H, J = 12.0 Hz), 3.40 (s, 3H), 3.31–3.25 (m, 2H), 2.96 (t, 2H, J = 12.0 Hz), 1.74–1.71 (m, 2H), 1.64–1.52 (m, 1H), 1.34–1.24 (m, 2H); MS (ESI), m/z: 435.17 $[M+H]^+$; HPLC purity = 98.9 %.

7-Allyl-8-(4-(hydroxymethyl)piperidin-1-yl)-3-methyl-1-(2,4,5-trifluorobenzyl)-1H-purine-2,6(3H,7H)-dione (**27**)

The title compound was prepared from 7-allyl-8-bromo-3-methyl-1-(2,4,5-trifluorobenzyl)-1H-purine-2,6(3H,7H)-dione (**7**) according to the procedure, for example **25**. White solid; Yield 68.3 %; mp 160–162 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.58–7.47 (m, 1H), 7.29–7.19 (m, 1H), 6.08–5.98 (m, 1H), 5.21 (d, 1H, J = 9.6 Hz), 5.10 (d, 1H, J = 17.6 Hz), 5.06 (s, 2H), 4.67 (d, 2H, J = 5.2 Hz), 4.52 (t, 1H, J = 5.2 Hz), 3.60 (d, 2H, J = 12.0 Hz), 3.39 (s, 3H), 3.32–3.25 (m, 2H), 2.95 (t, 2H, J= 12.0 Hz), 1.78–1.68 (m, 2H), 1.63–1.52 (m, 1H), 1.33–1.22 (m, 2H); MS (ESI), m/z: 464.25 $[M+H]^+$; HPLC purity = 98.2 %.

2-((8-(4-(Aminomethyl)piperidin-1-yl)-3-methyl-7-(3-methylbut-2-enyl)-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-1-yl)methyl)benzonitrile (**28**)

A mixture of **4** (1 mmol), tert-butyl piperidin-4-ylmethylcarbamate (1.1 mmol), and K_2CO_3 (2 mmol) in DMF (5 mL) was stirred at 75 °C for 8 h. The resulting mixture was cooled to room temperature. Then, water was added, and the resulting precipitate was separated by filtration. The precipitate was dried to give the crude N-tertbutyloxycarbonyl-protected intermediate. Next, a solution of the Boc-protected intermediate (1 mmol) in 2 mL of TFA/CH_2Cl_2 (v/v = 1/1) at 0 °C was stirred for 1 h and allowed to warm up to room temperature. After the reaction was completed, the solvent was removed under reduced pressure. The residue was slowly basified with 2 N NaOH and a solid was formed. The precipitate was collected by filtration and washed with water to afford the crude products, and the residue was purified by column chromatography on silica gel to afford target compound **28**. White solid; Yield 45.7 %; mp 138–140 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.83 (d, 1H, J = 7.6 Hz), 7.60 (t, 1H, J = 7.6 Hz), 7.44 (t, 1H, J = 7.6 Hz), 7.25 (d, 1H, J = 7.6 Hz), 5.35 (t, 1H, J = 6.0 Hz), 5.21 (s, 2H), 4.45 (d, 2H, J = 6.0 Hz), 3.56 (d, 2H, J = 11.6 Hz), 3.39 (s, 3H), 2.93 (t, 2H, J = 11.6 Hz), 2.49–2.42 (m, 2H), 1.87–1.79 (m, 2H), 1.71 (s, 3H), 1.68 (s, 3H), 1.53–1.36 (m, 1H), 1.31–1.20 (m, 2H); MS (ESI), m/z: 462.50 $[M+H]^+$; HPLC purity = 97.0 %.

8-(4-(Aminomethyl)piperidin-1-yl)-3-methyl-7-(3-methylbut-2-enyl)-1-(2,4,5-trifluorobenzyl)-1H-purine-2,6(3H,7H)-dione (**29**)

The title compound was prepared from 8-bromo-3-methyl-7-(3-methylbut-2-enyl)-1-(2,4,5-trifluorobenzyl)-1H-purine-2,6(3H,7H)-dione (**6**) according to the procedure, for example **28**. White solid; Yield 40.6 %; mp 125–127 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.60–7.48 (m, 1H), 7.30–7.17 (m, 1H), 5.33 (t, 1H, J = 6.4 Hz), 5.03 (s, 2H), 4.66 (d, 2H, J = 6.4 Hz), 3.55 (d, 2H, J = 12.0 Hz), 3.38 (s, 3H), 2.94 (t, 2H, J = 12.0 Hz), 2.63 (d, 2H, J = 6.4 Hz), 1.82–1.79 (m, 2H), 1.71 (s, 3H), 1.68 (s, 3H), 1.62–1.52 (m, 1H), 1.34–1.24 (m, 2H); MS (ESI), m/z: 491.42 $[M+H]^+$; HPLC purity = 97.7 %.

2-((7-Allyl-8-(4-(aminomethyl)piperidin-1-yl)-3-methyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-1-yl)methyl)benzonitrile (**30**)

The title compound was prepared from 2-((7-allyl-8-bromo-3-methyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-1-yl) methyl)benzonitrile (**5**) according to the procedure, for example **28**. White solid; Yield 53.7 %; mp 140–142 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.83 (d, 1H, J = 7.6 Hz), 7.62 (t, 1H, J = 7.6 Hz), 7.45 (t, 1H, J = 7.6 Hz), 7.24 (d, 1H, J = 7.6 Hz), 6.09–5.96 (m, 1H), 5.21 (d, 1H, J = 10.4 Hz), 5.20 (s, 2H), 5.11 (d, 1H, J = 17.2 Hz), 4.68 (d, 2H, J = 4.4 Hz), 3.61 (d, 2H, J = 12.0 Hz), 3.40 (s, 3H), 2.95 (t, 2H, J = 12.0 Hz), 2.47 (d, 2H, J = 6.4 Hz), 1.78–1.75 (m, 2H), 1.72–1.58 (m, 2H), 1.49–1.36 (m, 1H), 1.31–1.18 (m, 2H); MS (ESI), m/z: 434.10 $[M+H]^+$; HPLC purity = 96.8 %.

7-Allyl-8-(4-(aminomethyl)piperidin-1-yl)-3-methyl-1-(2,4,5-trifluorobenzyl)-1H-purine-2,6(3H,7H)-dione (**31**)

The title compound was prepared from 7-allyl-8-bromo-3-methyl-1-(2,4,5-trifluorobenzyl)-1H-purine-2,6(3H,7H)-dione (**7**) according to the procedure, for example **28**. White solid; Yield 51.5 %; mp 121–123 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.59-7.48 (m, 1H), 7.29–7.17 (m, 1H), 6.09–5.95 (m, 1H), 5.21 (d, 1H, J = 10.0 Hz), 5.10 (d, 1H, J = 17.2 Hz), 5.02 (s, 2H), 4.68 (d, 2H, J = 3.6 Hz), 4.42–3.95 (m, 1H), 3.60 (d, 2H, J = 12.0 Hz), 3.39 (s, 3H), 2.95 (t, 2H, J = 12.0 Hz), 2.56 (d, 2H, J = 6.4 Hz), 1.78–1.76 (m, 2H), 1.62–1.46 (m, 1H), 1.32–1.19 (m, 2H); MS (ESI), m/z: 463.10 $[M+H]^+$; HPLC purity = 97.7 %.

2-((8-(4-Aminopiperidin-1-yl)-3-methyl-7-(3-methylbut-2-enyl)-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-1-yl)methyl)benzonitrile (**32**)

A mixture of **4** (1 mmol), tert-butyl piperidin-4-ylcarbamate (1.1 mmol), and K_2CO_3 (2 mmol) in DMF (5 mL) was stirred at 75 °C for 6 h. The resulting mixture was cooled to room temperature. Then, water was added, and the resulting precipitate was separated by filtration. The precipitate was dried to give the crude N-tertbutyloxycarbonyl-protected intermediate. Next, a solution of the Boc-protected intermediate (1 mmol) in 2 mL of TFA/CH_2Cl_2 (v/v = 1/1) at 0 °C was stirred for 1 h and allowed to warm up to room temperature. After the reaction was completed, the solvent was removed under reduced pressure. The residue was slowly basified with 2 N NaOH and a solid was formed. The precipitate was collected by filtration and washed with water to afford the crude products, and the residue was purified by column chromatography on silica gel to afford target compound **32**. White solid; Yield 47.3 %; mp 167–169 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.84 (d, 1H, J = 7.6 Hz), 7.61 (t, 1H, J = 7.6 Hz), 7.42 (t, 1H, J = 7.6 Hz), 7.25 (d, 1H, J = 7.6 Hz), 5.33 (t, 1H, J = 6.4 Hz), 5.21 (s, 2H), 4.67 (d, 2H, J = 6.4 Hz), 3.52 (d, 2H, J = 12.0 Hz), 3.38 (s, 3H), 3.00 (t, 2H, J = 12.0 Hz), 2.92–2.88 (m, 1H), 1.87–1.84 (m, 2H), 1.71 (s, 3H), 1.68 (s, 3H), 1.53–1.40 (m, 2H); MS (ESI), m/z: 448.39 $[M+H]^+$; HPLC purity = 97.2 %.

7-Allyl-8-(4-aminopiperidin-1-yl)-3-methyl-1-(2,4,5-trifluorobenzyl)-1H-purine-2,6(3H,7H)-dione (**33**)

The title compound was prepared from 7-allyl-8-bromo-3-methyl-1-(2,4,5-trifluorobenzyl)-1H-purine-2,6(3H,7H)-dione (**7**) according to the procedure, for example **32**. White solid; Yield 46.8 %; mp 155–157 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.59–7.48 (m, 1H), 7.28–7.18 (m, 1H), 6.08–5.95 (m, 1H), 5.21 (d, 1H, J = 10.0 Hz), 5.09 (d, 1H, J = 17.6 Hz), 5.02 (s, 2H), 4.70 (d, 2H, J = 4.4 Hz), 3.57 (d, 2H, J = 12.0 Hz), 3.39 (s, 3H), 3.02 (t, 2H, J = 12.0 Hz), 3.01–2.98 (m, 1H), 1.86–1.84 (m, 2H), 1.50–1.48 (m, 2H); MS (ESI), m/z: 449.09 $[M+H]^+$; HPLC purity = 97.3 %.

2-((7-Allyl-8-(4-aminopiperidin-1-yl)-3-methyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-1-yl)methyl) benzonitrile (**34**)

The title compound was prepared from 2-((7-allyl-8-bromo-3-methyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-1-yl)methyl)benzonitrile (**5**) according to the procedure, for example **32**. White solid; Yield 57.2 %; mp 129–131 °C. ^{1}H NMR

(400 MHz, DMSO-d_6): δ 7.83 (d, 1H, J = 7.6 Hz), 7.62 (t, 1H, J = 8.0 Hz), 7.46 (t, 1H, J = 7.6 Hz), 7.24 (d, 1H, J = 8.0 Hz), 6.09–5.96 (m, 1H), 5.21 (d, 1H, J = 10.4 Hz), 5.20 (s, 2H), 5.10 (d, 1H, J = 17.2 Hz), 4.69 (d, 2H, J = 4.8 Hz), 3.55 (d, 2H, J = 12.0 Hz), 3.39 (s, 3H), 3.00 (t, 2H, J = 12.0 Hz), 2.81–2.72 (m, 1H), 1.83–1.76 (m, 2H), 1.72–1.64 (m, 2H), 1.43–1.27 (m, 2H); MS (ESI), m/z: 420.11 $[M+H]^+$; HPLC purity = 98.6 %.

3-Methyl-7-(3-methylbut-2-enyl)-8-(piperazin-1-yl)-1-(quinolin-2-ylmethyl)-1H-purine-2,6(3H,7H)-dione (**35**)

The title compound was prepared from 8-bromo-3-methyl-7-(3-methylbut-2-enyl)-1-(quinolin-2-ylmethyl)-1H-purine-2,6(3H,7H)-dione (**8**) according to the procedure, for example **14**. White solid; Yield 68.4 %; mp 150–152 °C. ^{1}H NMR (400 MHz, $CDCl_3$-d_6): δ 8.07 (d, 1H, J = 8.4 Hz), 8.03 (d, 1H, J = 8.4 Hz), 7.76 (d, 1H, J = 8.0 Hz), 7.66 (t, 1H, J = 8.0 Hz), 7.48 (t, 1H, J = 8.0 Hz), 7.30 (d, 1H, J = 8.8 Hz), 5.52 (s, 2H), 5.44 (t, 1H, J = 6.4 Hz), 4.74 (d, 2H, J = 6.4 Hz), 3.56 (s, 3H), 3.33–3.19 (m, 4H), 3.10–2.99 (m, 4H), 1.73 (s, 3H), 1.72 (s, 3H); MS (ESI), m/z: 460.18 $[M+H]^+$; HPLC purity = 96.4 %.

3-Methyl-7-(3-methylbut-2-enyl)-1-((2-oxo-1,2-dihydroquinolin-4-yl)-methyl)-8-(piperazin-1-yl)-1H-purine-2,6(3H,7H)-dione (**36**)

The title compound was prepared from 8-bromo-3-methyl-7-(3-methylbut-2-enyl)-1-((2-oxo-1,2-dihydroquinolin-4-yl) methyl)-1H-purine-2,6(3H,7H)-dione (**9**) according to the procedure, for example **14**. White solid; Yield 63.9 %; mp 267–269 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.81 (d, 1H, J = 8.0 Hz), 7.50 (t, 1H, J = 8.0 Hz), 7.36 (t, 1H, J = 8.0 Hz), 7.25 (d, 1H, J = 8.0 Hz), 6.20 (s, 1H), 5.49 (s, 2H), 5.43 (t, 1H, J = 6.0 Hz), 4.72 (d, 2H, J = 6.0 Hz), 3.57 (s, 3H), 3.32–3.22 (m, 4H), 3.10–3.00 (m, 4H), 1.74 (s, 3H), 1.73 (s, 3H); MS (ESI), m/z: 476.17 $[M+H]^+$; HPLC purity = 98.8 %.

4-((3-Methyl-7-(3-methylbut-2-enyl)-2,6-dioxo-8-(piperazin-1-yl)-2,3,6,7-tetrahydro-1H-purin-1-yl)methyl)benzonitrile (**37**)

The title compound was prepared from 4-((8-bromo-3-methyl-7-(3-methylbut-2-enyl)-2,6-dioxo-2,3,6,7-tetra hydro-1H-purin-1-yl)methyl)benzonitrile (**10**) according to the procedure, for example **14**. White solid; Yield 72.5 %; mp 190–192 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.77 (d, 2H, J = 8.0 Hz), 7.45 (d, 2H, J = 8.0 Hz), 5.32 (t, 1H, J = 6.4 Hz), 5.11 (s, 2H), 4.67 (d, 2H, J = 6.4 Hz), 3.38 (s, 3H), 3.16–3.07 (m, 4H), 2.85–2.77 (m, 4H), 2.39–2.38 (m, 1H), 1.70 (s, 3H), 1.67 (s, 3H); MS (ESI), m/z: 434.19 $[M+H]^+$; HPLC purity = 98.2 %.

3-Methyl-7-(3-methylbut-2-enyl)-8-(piperazin-1-yl)-1-(4-(trifluoromethyl)-benzyl)-1H-purine-2,6(3H,7H)-dione (**38**)

The title compound was prepared from 8-bromo-3-methyl-7-(3-methylbut-2-enyl)-1-(4-(trifluoromethyl)benzyl)-1H-purine-2,6(3H,7H)-dione (**11**) according to the procedure, for example **14**. White solid; Yield 74.8 %; mp 136–138 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.77 (d, 2H, J = 7.6 Hz), 7.45 (d, 2H, J = 7.6 Hz), 5.33 (t, 1H, J = 5.2 Hz), 5.12 (s, 2H), 4.68 (d, 2H, J = 5.2 Hz), 3.38 (s, 3H), 3.17–3.06 (m, 4H), 2.87–2.77 (m, 4H), 1.71 (s, 3H), 1.67 (s, 3H); MS (ESI), m/z: 477.21 $[M+H]^+$; HPLC purity = 98.0 %.

Methyl-4-((3-methyl-7-(3-methylbut-2-enyl)-2,6-dioxo-8-(piperazin-1-yl)-2,3,6,7-tetrahydro-1H-purin-1-yl)methyl)benzoate (**39**)

The title compound was prepared from Methyl-4-((8-bromo-3-methyl-7-(3-methylbut-2-enyl)-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-1-yl)methyl)benzoate (**12**) according to the procedure, for example **14**. White solid; Yield 72.6 %; mp 170–172 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.90 (d, 2H, J = 7.6 Hz), 7.39 (d, 2H, J = 7.6 Hz), 5.33 (t, 1H, J = 5.2 Hz), 5.10 (s, 2H), 4.68 (d, 2H, J = 5.2 Hz), 3.83 (s, 3H), 3.38 (s, 3H), 3.18–3.05 (m, 4H), 2.89–2.76 (m, 4H), 2.41–2.28 (m, 1H), 1.71 (s, 3H), 1.67 (s, 3H); MS (ESI), m/z: 467.20 $[M+H]^+$; HPLC purity = 98.7 %.

2-((8-(3-Aminopiperidin-1-yl)-3-methyl-7-(3-methylbut-2-enyl)-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-1-yl)methyl)benzonitrile (**40**)

A solution of racemic-3-Boc–aminopiperidine (1.1 mmol), **4** (1 mmol), K_2CO_3 (2 mmol) in DMF (5 mL) was stirred at 75 °C for 6 h. Then, water was added, and the resulting precipitate was separated by filtration. The precipitate was dried to give the crude N-tertbutyloxycarbonyl-protected intermediate. Next, a solution of the Boc-protected intermediate (1 mmol) in 2 mL of TFA/CH_2Cl_2 (v/v = 1/1) at 0 °C was stirred for 1 h and allowed to warm up to room temperature. After the reaction was completed, the solvent was removed under reduced pressure. The residue was slowly basified with 2N NaOH and a solid was formed. The precipitate was collected by filtration and washed with water to afford the crude products, and the residue was further purified by column chromatography on silica gel to afford target racemic compound **40**. White solid; Yield 48.3 %; mp 202–204 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.94–8.37 (m,

2H), 7.84 (d, 1H, J = 7.6 Hz), 7.63 (t, 1H, J = 7.6 Hz), 7.46 (t, 1H, J = 7.6 Hz), 7.26 (d, 1H, J = 7.6 Hz), 5.34 (t, 1H, J = 6.0 Hz), 5.22 (s, 2H), 4.81–4.76 (m, 1H), 4.71–4.65 (m, 1H), 3.64–3.62 (m, 1H), 3.40 (s, 3H), 3.28–3.25 (m, 1H), 3.18–3.13 (m, 1H), 3.00–2.88 (m, 1H), 2.10–1.96 (m, 1H), 1.95–1.84 (m, 1H), 1.73 (s, 3H), 1.69 (s, 3H), 1.66–1.58 (m, 3H); MS (ESI), m/z: 448.19 $[M+H]^+$; HPLC purity = 98.5 %.

4-((8-(3-Aminopiperidin-1-yl)-3-methyl-7-(3-methylbut-2-enyl)-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-1-yl)methyl)benzonitrile (**41**)

The racemic compound **41** was prepared from 4-((8-bromo-3-methyl-7-(3-methylbut-2-enyl)-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-1-yl)methyl)benzonitrile (**10**) according to the procedure, for example **40**. White solid; Yield 53.6 %; mp 220–222 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.77 (d, 2H, J = 7.6 Hz), 7.45 (d, 2H, J = 7.6 Hz), 5.33 (t, 1H, J = 10.0 Hz), 5.10 (s, 2H), 4.68 (d, 2H, J = 10.0 Hz), 3.55–3.44 (m, 2H), 3.38 (s, 3H), 2.90–2.73 (m, 2H), 2.64–2.59 (m, 1H), 1.91–1.80 (m, 1H), 1.80–1.73 (m, 1H), 1.71 (s, 3H), 1.67 (s, 3H), 1.64–1.49 (m, 1H), 1.23–1.09 (m, 1H); MS (ESI), m/z: 448.18 $[M + H]^+$; HPLC purity = 98.9 %.

Methyl-4-((8-(3-aminopiperidin-1-yl)-3-methyl-7-(3-methylbut-2-enyl)-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-1-yl)methyl)benzoate (**42**)

The racemic compound **42** was prepared from Methyl-4-((8-bromo-3-methyl-7-(3-methylbut-2-enyl)-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-1-yl)methyl)benzoate (**12**) according to the procedure, for example **40**. White solid; Yield 52.5 %; mp 183–185 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.22–8.04 (m, 2H), 7.90 (d, 2H, J = 8.0 Hz), 7.40 (d, 2H, J = 8.0 Hz), 5.37–5.29 (m, 1H), 5.11 (s, 2H), 4.80–4.73 (m, 1H), 4.70–4.61 (m, 1H), 3.83 (s, 3H), 3.59–3.57 (m, 1H), 3.39 (s, 3H), 3.38–3.36 (m, 2H), 3.28–3.25 (m, 1H), 3.13–3.02 (m, 1H), 2.96–2.91 (m, 1H), 2.05–1.97 (m, 1H), 1.93–1.82 (m, 1H), 1.73 (s, 3H), 1.68 (s, 3H), 1.63–1.52 (m, 1H); MS (ESI), m/z: 481.18 $[M+H]^+$; HPLC purity = 98.2 %.

8-(3-Aminopiperidin-1-yl)-1-(benzo[d]thiazol-2-ylmethyl)-3-methyl-7-(3-methylbut-2-enyl)-1H-purine-2,6(3H,7H)-dione (**43**)

The racemic compound **43** was prepared from 1-(benzo[d]thiazol-2-ylmethyl)-8-bromo-3-methyl-7-(3-methylbut-2-enyl)-1H-purine-2,6(3H,7H)-dione (**13**) according to the procedure, for example **40**. White solid; Yield 58.5 %; mp 165–167 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.05 (d, 1H, J = 7.6 Hz), 7.95 (d, 1H, J = 8.4 Hz), 7.51 (t, 1H, J = 8.0 Hz), 7.44 (t, 1H, J = 8.0 Hz), 6.87–6.27 (m, 2H), 5.46 (s, 2H), 5.34–5.32 (m, 1H), 4.81–4.60 (m, 2H), 3.62–3.52 (m, 1H), 3.42 (s, 3H), 3.21–3.11 (m, 1H), 3.00–2.87 (m, 2H), 2.76–2.65 (m, 1H), 2.03–1.90 (m, 1H), 1.89–1.72 (m, 1H), 1.72 (s, 3H), 1.68 (s, 3H), 1.64–1.52 (m, 1H), 1.51–1.38 (m, 1H); MS (ESI), m/z: 480.19 $[M + H]^+$; HPLC purity = 98.2 %.

In vitro inhibition of DPP-IV

DPP-IV inhibition was measured using DPP-IV inhibitor screening assay kit (Cayman Chemical Company, Item No. 700210) following the manufacturer's instructions. Briefly, solutions of test compounds at varying concentrations were prepared in dimethyl sulfoxide (DMSO) and then diluted into assay buffer. Human recombinant DPP-IV was added to the dilutions and pre-incubated for 30 min at 37 °C before the reaction was initiated by the addition of fluorogenic substrate solution AMC (Gly-Pro-Aminomethylcoumarin). The kinetics of the reaction was monitored (excitation at 380 nm, emission at 460 nm). Inhibition constants (IC_{50}) were calculated from enzyme progress curves using standard mathematical models.

In vitro inhibition of DPP-8 and DPP-9

DPP-8 and DPP-9 inhibitions were measured using fluorogenic DPP-8 assay kit and fluorogenic DPP-9 assay kit (BPS Bioscience, San Diego, CA, USA) following the manufacturer's instructions. Briefly, solutions of test compounds in varying concentrations were prepared in dimethyl sulfoxide (DMSO) and then diluted into assay buffer. Human recombinant DPP-8 or DPP-9 was added to the dilutions and pre-incubated for 10 min at 22 °C before the reaction was initiated by the addition of fluorogenic DPP substrate. The kinetics of the reaction was monitored (excitation at 380 nm, emission at 460 nm). Inhibition constants (IC_{50}) were calculated from enzyme progress curves using standard mathematical models.

Oral glucose tolerance test in diet-induced obesity (DIO) Mice

6-Week-old male C57BL/6J mice were fed a high-fat diet (HFD, Research Diets) ad libitum for 8 weeks. The experiment was performed in DIO mice after 18–21 hours of fasting. Blood glucose taken from the tail tip was measured using a glucose meter (Accu-Chek, Roche). Following the measurement of basal blood glucose concentration, saline, test compounds (5 mg/kg) or metformin (150 mg/kg) was injected into the peritoneal cavity of mice in different groups, respectively. After 30 min, 2.0 g/kg glucose was adminis-

trated by oral to each group and this time point was set as 0 min. The blood glucose was determined at 15, 30, 45, 60, 90 min after glucose challenged. The blood glucose excursion profile from $t = 0$ min to $t = 90$ min was used to integrate an area under the curve (AUC) for each group.

Animal model and treatment of diet-induced obesity

C57BL/6J mice were obtained from Western China Experimental Animal Center and housed individually in a room maintained at 25 °C on a light/dark schedule. For DIO mice, 6-week-old male C57BL/6J mice were fed a high-fat diet (HFD, Research Diets) ad libitum for 8 weeks. Then, these animals were assigned to four groups randomly consisting of 10 mice each. 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 60.0 % of calories from fat, 20.0 % from protein and 20.0 % from carbohydrates. HFD+Met (150 mg/kg/day), HFD+**40** (5 mg/kg/day) and HFD+**43** (5 mg/kg/day) in PEG 400 or vehicle were orally administered per day for 5 weeks. Body weight and food intake were measured per day. After all animals were sacrificed, serum levels of markers were analyzed. The percentages were calculated by (V_{HFD} − Vtreatment)/ $V_{HFD} \times 100$ %.

Supplementary Data ^{1}HNMR, ^{13}C analysis of synthesized compounds.

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