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**Graphical Abstract**

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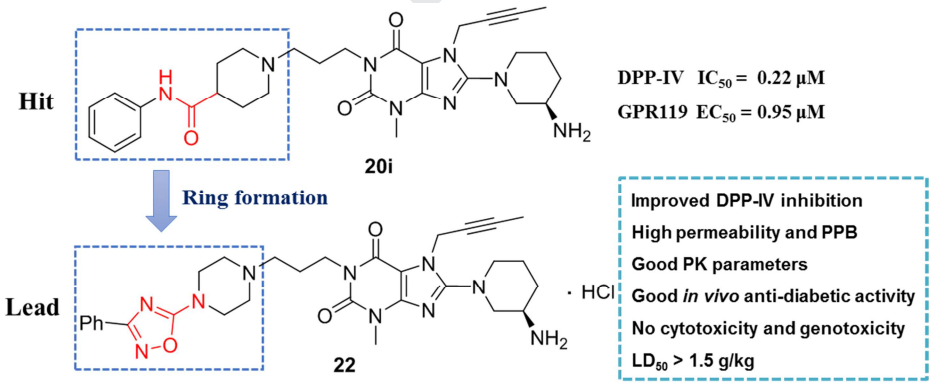
*State Key Laboratory of Bioactive Substance and Function of Natural Medicines & Beijing Key Laboratory of Active Substance Discovery and Druggability Evaluation, Institute of Materia Medica, Peking Union Medical College and Chinese Academy of Medical Sciences, Beijing 100050, P. R. China*



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# The optimization of xanthine derivatives leading to HBK001 hydrochloride as a potent dual ligand targeting DPP-IV and GPR119

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## Abstract

A series of xanthine compounds derived from the previous hit **20i** with modification on the terminal side chain was discovered through ring formation strategy. Systematic optimization of the compounds with rigid heterocycles in the hydrophobic side chain led to the new lead compound HBK001 (**21h**) with the improved DPP-IV inhibition and moderate GPR119 agonism activity *in vitro*. As a continuing work to further study the PK and PD profiles, **21h** and its hydrochloride (**22**) were synthesized on grams scale and evaluated on the ADME/T and oral glucose tolerance test (OGTT) in ICR mice. Compound **22** showed the improved bioavailability and blood glucose-lowering effect *in vivo* compared to its free base **21h** probably attributed to its improved solubility and permeability. The preliminary toxicity studies on compound **22** exhibited that the result of mini-Ames was negative and the preliminary acute toxicity $LD_{50}$ in mice was above 1.5 g/kg, while it showed moderate inhibition on hERG channel with $IC_{50}$ 4.9 μM maybe due to its high lipophilicity. These findings will be useful for the future drug design for more potent and safer dual ligand targeting DPP-IV and GPR119 for the treatment of diabetes.

## Key words

DPP-IV; GPR119; xanthine compounds; HBK001; druggability;

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[1] These authors contributed equally to this article.

## 1. Introduction

Type 2 diabetes mellitus (T2DM) is a progressive chronic disease characterized by insulin resistance and defective insulin secretion from the pancreas [1]. During the development of T2DM, pancreatic β-cell function gradually declines resulted in a severe deduction of insulin secretion [2]. Glucagon like peptide-1 (GLP-1) is one of the major incretins released from intestinal enteroendocrine L cells and plays an important role in nutrient metabolism and glucose homeostasis [3, 4]. As GLP-1 is rapidly degraded by dipeptidyl peptidase IV (DPP-IV), DPP-IV inhibitors can prolong the half-life of active GLP-1, which promotes glucose-stimulated insulin secretion and decreases glucose [5].

Recently, along with the successful commercial launch of GLP-1 analogues and DPP-IV inhibitors, intensive interest in the development of T2DM therapeutics has been stimulated on the targets which enhance insulin secretion in a glucose-dependent manner in order to minimize the risk of hypoglycemia [6]. On the other hand, G-protein-coupled receptor 119 (GPR119) has been emerged as one of the most exciting targets for the treatment of T2DM. GPR119 agonists mediate a unique dual elevation of both insulin and GLP-1, followed by a reduction in blood glucose level [7]. Representative launched DPP-IV inhibitors such as Sitagliptin, Linagliptin, Tenegliptin and GPR119 agonists under research such as AR231453, MBX-2982 are depicted in **Fig. 1**.

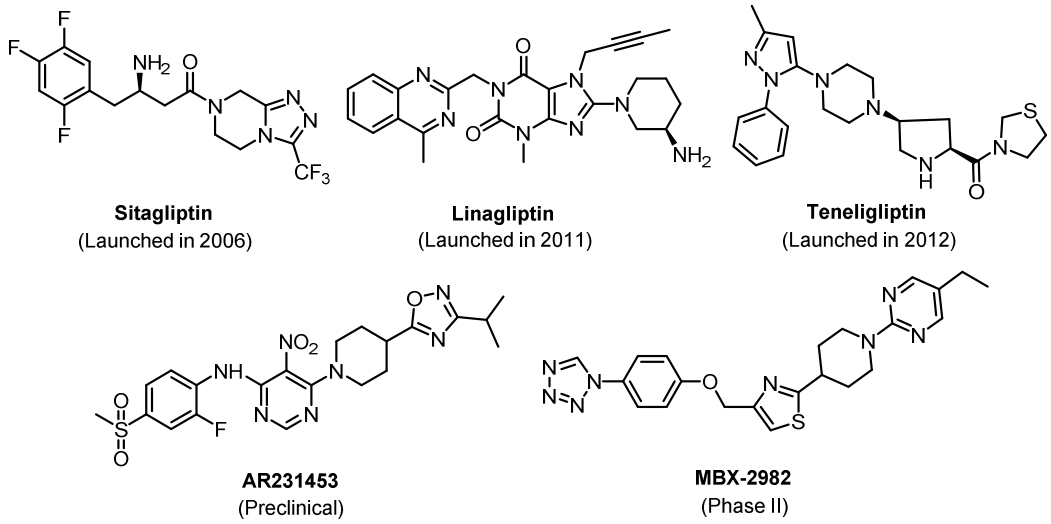



**Fig. 1.** Structures of representative DPP-IV inhibitors and GPR119 agonists

Therefore, based on the biological effects of GLP-1, the synergistic effects between DPP-IV inhibition and GPR119 agonism may bring advantages in blood glucose control. Indeed, in animal experiments, combining a DPP-IV inhibitor with a GPR119 agonist significantly increased the plasma active GLP-1 levels and improved glucose clearance [8, 9]. Inspired by the promising results, the proof of concept hit **20i** with jointed DPP-IV inhibition and GPR119 agonism activity was discovered by our group through systematic structural modification based on the pharmacophore merged strategy [10]. The multi-target ligands with the lower risk of drug-drug interactions and simplified clinical development could offer a new therapeutic option in the treatment of type 2 diabetes [11-14].

In order to further improve the dual DPP-IV and GPR119 activities, the amide in the hydrophobic side chain of **20i** was converted to various rigid heterocycles through ring formation strategy (**Fig. 2**). Among them, compound HBK001 **(21h)** with oxadiazole side chain can both inhibit DPP-IV and activate GPR119 *ex* and *in vivo*. Furthermore, HBK001 **(21h)** can improve islet morphology, increase β-cell proliferation and up-regulate genes involved in improved β-cell function [15].

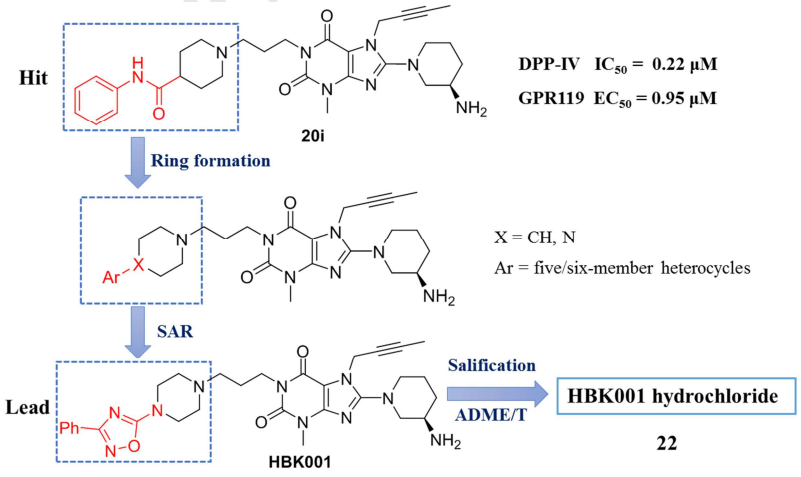


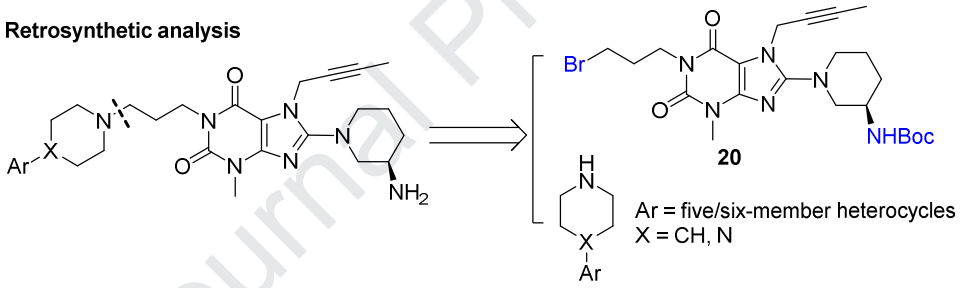

**Fig. 2.** Structure optimization through the ring formation strategy

Herein, we present the synthesis, SARs and ADME/T of HBK001 (**21h**) and its derivatives. All the compounds exhibited good DPP-IV inhibitory activities and **21h** showed better DPP-IV inhibition and comparative GPR119 agonism activity than the hit **20i**. Further druggability results revealed that HBK001 hydrochloride (**22**) showed

improved bioavailability and glucose tolerance compared to its free base. The further modification based on the identified compound **22** is ongoing for the discovery of dual ligand drug candidate targeting DPP-IV and GPR119 for the treatment type 2 diabetes.

## 2. Chemistry

The target xanthine derivatives can be conveniently afforded by reacting xanthine intermediate **20** with piperidyl/piperazinyl intermediates through retrosynthetic analysis (**Scheme 1**). The 1,3-dibromopropane was used as the key precursor reagent to construct the linker between the xanthine and piperidyl/piperazinyl scaffolds. A series of five/six-member heterocycles, derived from the privileged scaffold of DPP-IV inhibitors and GPR119 agonists, was involved in the terminal of the aromatic side chain.



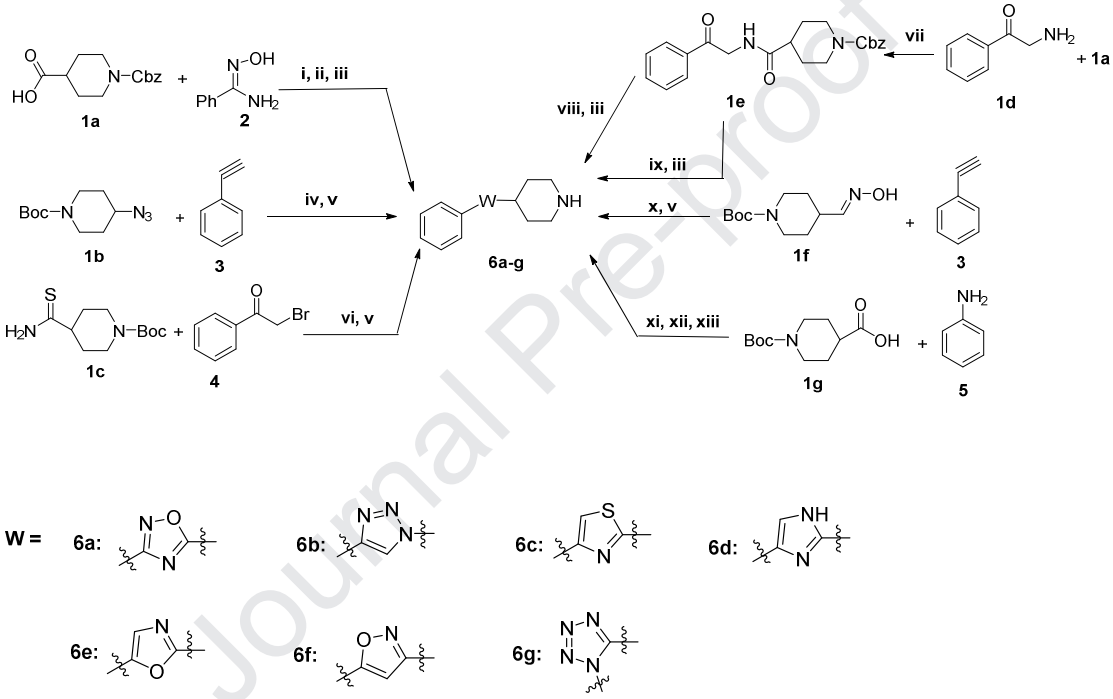

**Scheme 1**. Retrosynthetic analysis of the target compounds

The synthesis of key intermediates **6a-g**, **16a-f** and **19a-f** containing heterocycles is outlined in **Scheme 2-4**. The xanthine intermediate **20** is synthesized according to our previous work [10]. The general synthetic procedures of the target compounds **21a-s** and a hydrochloride **22** are illustrated in **Scheme 5**.

2.1 Synthesis of the piperidyl intermediates **6a-g**

The *N*-Cbz piperidine-4-carboxylic acid **1a** was treated with oxalyl chloride followed by refluxing with *N'*-hydroxybenzimidamide **2** in toluene and deprotection of Cbz to afford the oxadiazole intermediate **6a** [16]. The triazole piperidyl **6b** was obtained through the click reaction between the azide **1b** and phenylacetylene **3** in the presence of CuI and DIPEA [17]. The thiazole **6c** was formed by the heterocyclization

of *N*-Boc piperidine-4-thioformamide **1c** and α-bromoacetophenone **4** followed by the deprotection of Boc group. The intermediate **1e**, prepared from the amidation of **1a** and **1d**, reacted with $AcONH_4$ in the solvent of AcOH to produce imidazole **6d** while the treatment of **1e** with $POCl_3$ in the presence of pyridine generated oxazole **6e** [18]. The phenylacetylene **3** was treated with **1f** in the presence of potassium peroxymonosulfate to form isoxazole **6f**. Tetrazole **6g** was obtained by the amidation of *N*-Boc protected piperidine-4-carboxylic acid **1g** and aniline **5** followed by heterocyclization and deprotection [19].

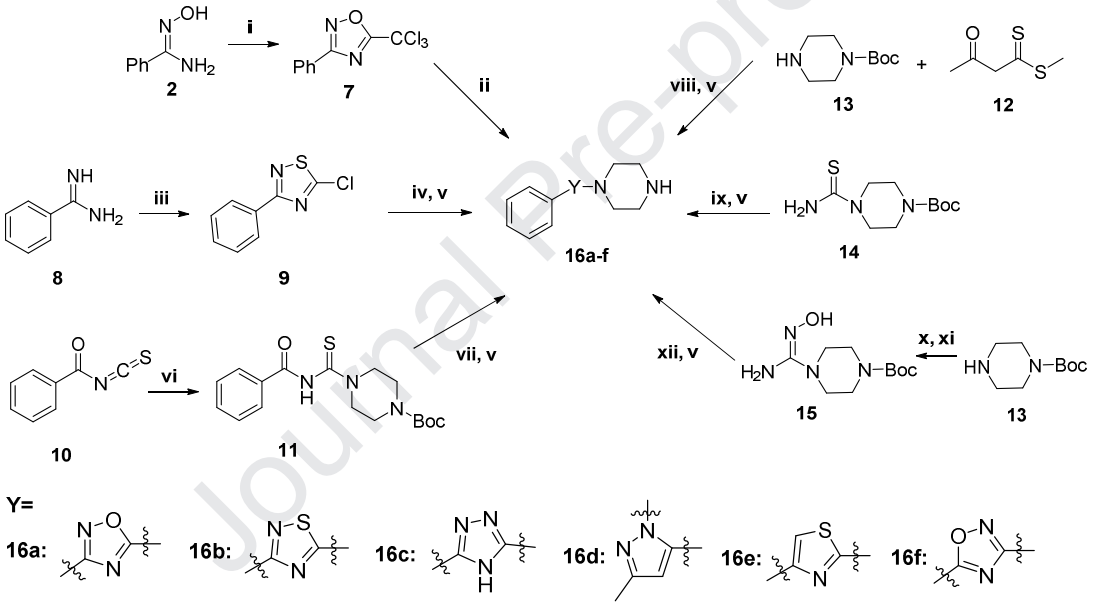



**Scheme 2.** Synthesis of the intermediates **6a-g.** Reagents and conditions: i. Oxalyl chloride, DMF, DCM, r.t., 3h; ii. Toluene, reflux, 4h; iii. TMSI, DCM, 0°C - r.t., 4h; iv. CuI, DIPEA, DCM, r.t., 2h; v. TFA, DCM, r.t., 2h; vi. $NaHCO_3$, ethanol, reflux, 3h; vii. CDI, DIPEA, DCM, 0°C - r.t., 12h; viii. $AcONH_4$, AcOH, reflux, 5h; ix. $POCl_3$, pyridine, r.t., 2h; x. KCl, $HKO_6S$, $H_2O$, 0°C - r.t., 12h; xi. EDCI, HOBt, TEA, acetonitrile, r.t., 5h; xii. TMS-$N_3$, $Ph_3P$, DIAD, r.t. 6 days then reflux 1h; xiii. 7N HCl-EA, r.t., 1h.

## 2.2 Synthesis of the piperazinyl intermediates **16a-f**

The *N'*-hydroxybenzimidamide **2** was treated with trichloroacetic anhydride followed by the substitution with piperazine to form the oxadiazole intermediate **16a** [20]. The treatment of benzimidamide **8** with (trichloromethyl)sulfenyl chloride in the presence of NaOH generated intermediate **9**. The thiadiazole **16b** was formed by the

substitution of **9** followed by the deprotection of Boc group [21]. Reacting **10** with Boc-piperazine produced intermediate **11** [22]. The triazole **16c** was obtained by the heterocyclization of **11** and hydrazine monohydrate followed by the deprotection [23]. The methyl 3-oxobutanedithioate **12** was treated with Boc-piperazine followed by the heterocyclization with phenylhydrazine and deprotection of Boc to afford the pyrazole **16d**. The thiazole **16e** was formed by the heterocyclization of **14** and 2-bromoacetophenone followed by the deprotection [24]. The intermediate **15** was prepared from the substitution of cyanogen bromide followed by the addition of hydroxylamine. The treatment of **15** with benzoic acid and CDI under refluxing condition followed by the deprotection afforded intermediate **16f** [25].

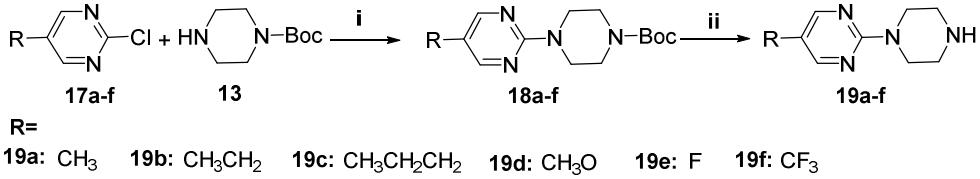



**Scheme 3.** Synthesis of the intermediates **16a-f.** Reagents and conditions: i. Trichloroacetic anhydride, toluene, reflux, 10h; ii. Anhydrous piperazine, DMF, r.t., 6h. iii. (Trichloromethyl)sulfenyl chloride, NaOH, DCM, 0°C - r.t., 3h; iv. 1-Boc-piperazine, TEA, DMF, r.t., 12h; v. TFA, DCM, r.t., 2h. vi. 1-Boc-piperazine, DCM, 0°C - r.t., 2h; vii. Hydrazine monohydrate, $CHCl_3$, reflux, 4h; viii. Phenylhydrazine, AcOH, 4A molecular sieve, dry ethanol, reflux, 15h; ix. 2-Bromoacetophenone, $NaHCO_3$, ethanol, reflux, 4h. x. Cyanogen bromide, $NaHCO_3$, DCM/$H_2O$, r.t., 2h; xi. 50% $NH_2$-OH aqueous solution, EtOH, reflux, 6h; xii. Benzoic acid, CDI, DMF, 120°C, 8h.

2.3 Synthesis of the pyrimidyl intermediates **19a-f**

The reactions of commercially available starting material **17a-f** with

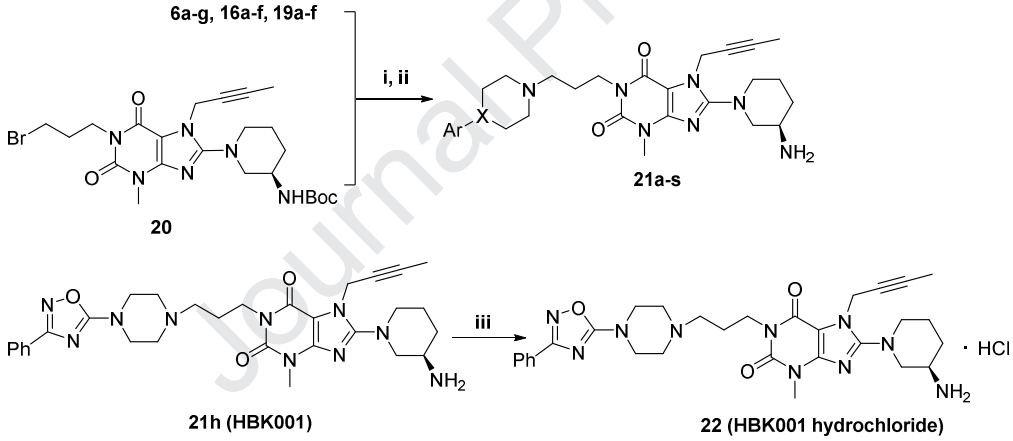
Boc-piperazine **13** in the presence of $K_2CO_3$ produced **18a-f** [26]. Intermediates **19a-f** were obtained by the *N*-Boc deprotection of **18a-f**.




**Scheme 4.** Synthesis of the intermediates **19a-f.** Reagents and conditions: i. TEA, ethanol, reflux, 6h; ii. TFA, DCM, r.t., 3h.

2.4 General synthesis of the target compounds **21a-s** and **22**

Finally, the nucleophilic substitution of xanthine intermediate **20** [10] with the corresponding intermediates **6a-g**, **16a-f** and **19a-f** in the presence of DIPEA, followed by deprotection of Boc group, provided the target compounds **21a-s** respectively. The HBK001 hydrochloride (**22**) was formed with a solution of HCl/EtOH via salification of its free base **21h**.




**Scheme 5.** General synthesis of the target compounds **21a-s** and **22.** Reagents and conditions: i. DIPEA, DMF, 80°C, 10h; ii. TFA, DCM, r.t., 2h; iii. HCl/EtOH, r.t., 3h.

2.5 Single-crystal X-ray structure of compound **21h**

The configuration of **21h** was confirmed by an X-ray crystal structure (CCDC 1943980, Fig. 3). The X-ray analysis data demonstrates the exclusive formation of (*R*)-3-aminopiperidine at C-8. No intramolecular hydrogen bond was found and the hydrophobic phenyl oxadiazole side chain was linear as expected, which was in accordance with the pharmacophore feature for the GPR119 agonist [7].

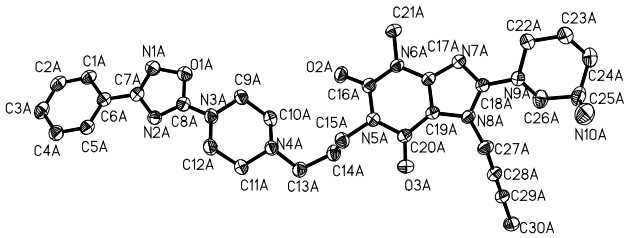



**Fig. 3.** X-ray crystallographic structure of compound **21h**

## 3. Results and discussion

The target compounds were evaluated *in vitro* for their inhibition of DPP-IV, DPP-8/9 and agonism activities of GPR119. The inhibition against DPP-IV as $IC_{50}$ values and the agonism to GPR119 as $EC_{50}$ values of the selected compounds were summarized in **Table 1-2** with sitagliptin and AR231453 as the positive controls. The relative activity percentages of the corresponding reference compounds are normalized to the activity of AR231453 at the same concentration, and the $EC_{50}$ value refers to the concentration of the indicated compound reaching half potency of GPR119 agonism itself [10].

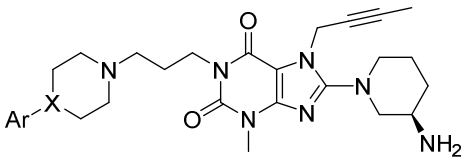
### 3.1 Hit optimization through ring formation strategy

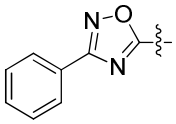
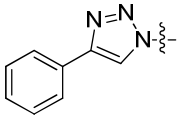
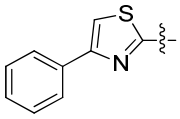
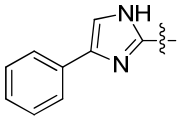
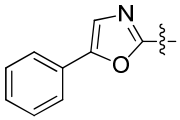
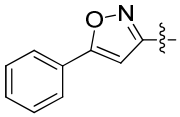
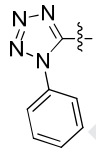
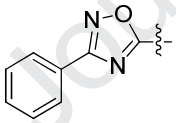
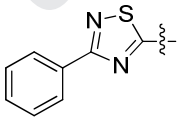
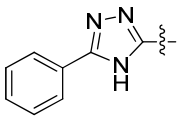
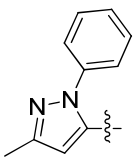
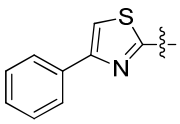
Our previous work revealed that the linker length of 3 methylene units and (*R*)-3-aminopiperidine were the crucial constituents for the activities [10]. Hence, our continuous systematic modification was focused on the terminal side chain through ring formation strategy based on our previous hit **20i**. The amide group was directly converted to various five-member heterocycles **21a-g** (**Table 1**). To our delight, the compound **21a-g** with rigid heterocycles retained good potency of DPP-IV with $IC_{50}$ values ranged from 0.044 μM to 0.27 μM. In the meanwhile, the compounds **21c-e** which have the same heteroatom position exhibited good GPR119 agonism above 60% activity of AR231453 at 1 μM. In particular, compound **21b** and **21d** with the triazole and imidazole units demonstrated much better DPP-IV inhibitory activities than **20i** (DPP-IV, $IC_{50}$ = 0.22 μM) [10], which offered 5-fold improvement in potency. The results indicated that the introduction of additional conformational rigidity led to the improved DPP-IV inhibitory activities. Specifically, not only did compound **21d**

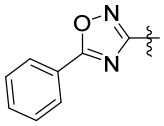
display potent DPP-IV inhibition ($IC_{50}$ = 0.044 μM), it also exhibited good GPR119 agonism activity ($EC_{50}$ = 0.6 μM). Meanwhile, these compounds showed excellent DPP-IV selectivity over DPP-8/9, which is one key requirement due to the safety concern.

Inspired by the improved DPP-IV inhibition from 0.22 μM (**20i**) to 0.044 μM (**21d**) through the ring formation strategy, other versatile five-member heterocycles with the piperazine linker were conducted (**21h-m**). It turned out that the installation of polar nitrogen atom led to the improved activities against DPP-IV exemplified by compound **21a** with piperidine linker ($IC_{50}$ = 0.23 μM) versus **21h** with piperazine linker ($IC_{50}$ =0.04 μM), and similarly compound **21c** ($IC_{50}$ = 0.19 μM) versus **21l** ($IC_{50}$ = 0.059 μM). Especially, compound **21h** and **21l** also showed the good potency of GPR119 compared to the positive control AR231453 *in vitro*. When the oxygen atom in the oxadiazole in compound **21h** was replaced by sulfur and nitrogen atom, the corresponding compounds **21i** and **21j** decreased DPP-IV potency to 0.36 μM and 0.076 μM, respectively. Among them, the GPR119 agonism of compound **21j** was disappeared. In addition, the position exchange of oxygen and nitrogen in the oxadiazole (**21m**) resulted in the decreased DPP-IV inhibitory activity and maintained GPR119 activity compared to compound **21h**. The introduction of 1-(3-methyl-1-phenyl-1*H*-pyrazol-5-yl)piperazine unit which selected from the side chain of Teneligliptin afforded the desirable DPP-IV potency, but lost GPR119 agonism maybe due to the loss of structural linearity (**21k**). To our delight, the compounds **21h** and **21i** could maintain comparable potency toward GPR119 ($EC_{50}$ = 1.40 and 1.93 μM), especially, compound **21h** displayed very potent DPP-IV inhibitory activity.

**Table 1.** Activity of the target compounds with five-member heterocycles against DPP-IV, DPP-8/9 and GPR119



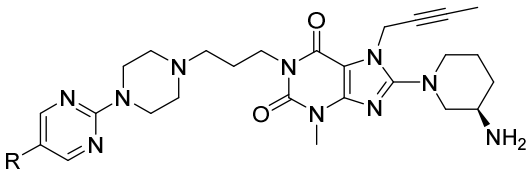
| Compds. | Ar | X | DPP-IV | | DPP-8/9 (10 μM) | GPR119 | |
|---|---|---|---|---|---|---|---|
| | | | %Inhibition[a] (10 μM) | $IC_{50}$[b] (μM) | | %Activity[c] (1 μM) | $EC_{50}$[d] (μM) |
| **21a** |  | CH | 91.8 | 0.23 | - | 55 | - |
| **21b** |  | CH | 98.8 | 0.047 | 0 | 47 | - |
| **21c** |  | CH | 79.5 | 0.19 | 2.2 | 83 | 3.13 |
| **21d** |  | CH | 89.2 | 0.044 | 5.1 | 65 | 0.6 |
| **21e** |  | CH | 97.3 | 0.12 | 3.3 | 80 | 0.83 |
| **21f** |  | CH | 89.2 | 0.19 | 3.3 | 49 | - |
| **21g** |  | CH | 89.0 | 0.27 | 4.6 | 53 | - |
| **21h** |  | N | 97.8 | 0.04 | 0 | 58 | 1.40 |
| **21i** |  | N | 86.5 | 0.36 | 1.6 | 81 | 1.93 |
| **21j** |  | N | 94.5 | 0.076 | 7.3 | 38 | - |
| **21k** |  | N | 82.9 | 0.083 | 15.4 | 42 | - |
| **21l** |  | N | 86.3 | 0.059 | 0 | 56 | 0.65 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **21m** |  | N | 87.1 | 0.68 | - | 89 | 1.45 |
| **20i**[e] | | | 93.6 | 0.22 | 36.8 | 81.5 | 0.95 |
| **Sitagliptin** | | | 99.0 | 0.0084 | - | - | - |
| **AR231453** | | | - | - | - | 100 | 0.64 |

[a]Maximum % inhibition against DPP-IV. [b]Concentration resulting in 50% maximum inhibition of DPP-IV. [c]Relative activity percentage was normalized to the activation fold of AR231453. [d]Concentration resulting in 50% maximum activation activity of GPR119. [e]The data were cited from our previous result [10].

Subsequently, we concentrated our efforts on the further modification of GPR119 pharmacophore. Attracted by the GPR119 agonist MBX-2982 in phase II clinical stage, its substituted pyrimidine unit was chosen as the terminal side chain to form the new target compounds **21n-s** (**Table 2**). It revealed that all the xanthine compounds containing pyrimidine could maintain GPR119 agonism above 60% activity at **1** μM with $EC_{50}$ values ranged from 1.41 μM to 4.02 μM. Among them, compound **21r** ($EC_{50}$ = 1.41 μM) with fluorine substituted pyrimidine side chain exhibited better potency than **21o** ($EC_{50}$ = 3.99 μM) containing ethyl pyrimidine side chain like MBX-2982. The bulky hydrophobic propyl pyrimidine compound **21p** showed better activity than compounds **21n** and **21o** toward GPR119 ($EC_{50}$ = 1.85 μM vs 2.56 μM, 3.99 μM). The compounds with electron-donating group (**21n** and **21q**) displayed better GPR119 agonism than the compound with strong electron-withdrawing group (**21s**) ($EC_{50}$ = 2.56 μM, 2.26 μM vs 4.02 μM). In addition, the substitution of *N*-benzyl amide with pyrimidine could maintain the DPP-IV inhibitory activity with $IC_{50}$ values ranged from 0.35 μM to 0.74 μM compared to the previous hit **20i** ($IC_{50}$ = 0.22 μM). Although the target compounds with pyrimidine side chain possessed moderate to good GPR119 agonism activity compared to the compounds containing five-member heterocycles, that these compounds decreased DPP-IV inhibitor activity to some extent was unanticipated.

**Table 2.** Activity of the target compounds with six-member heterocycles against DPP-IV and GPR119



| Compds. | R | DPP-IV | | GPR119 | |
|---|---|---|---|---|---|
| | | %Inhibition[a] (10 μM) | $IC_{50}$[b] (μM) | %Activity[c] (1 μM) | $EC_{50}$[d] (μM) |
| **21n** | $CH_3$ | 91.2 | 0.66 | 60 | 2.56 |
| **21o** | $CH_3CH_2$ | 86.3 | 0.61 | 85 | 3.99 |
| **21p** | $CH_3CH_2CH_2$ | 89.2 | 0.59 | 84 | 1.85 |
| **21q** | $CH_3O$ | 90.5 | 0.43 | 79 | 2.26 |
| **21r** | F | 93.4 | 0.35 | 78 | 1.41 |
| **21s** | $CF_3$ | 88.0 | 0.74 | 90 | 4.02 |
| **Sitagliptin** | | 91.7 | 0.018 | - | - |
| **AR231453** | | - | - | 100 | 0.64 |

[a]Maximum % inhibition against DPP-IV. [b]Concentration resulting in 50% maximum inhibition of DPP-IV. [c]Relative activity percentage was normalized to the activation fold of AR231453. [d]Concentration resulting in 50% maximum activation activity of GPR119.

3.2 Lead identification through preliminary druggability evaluation

To identify the metabolic and toxic liabilities of the novel xanthine series, compounds **21b**, **21d**, **21h** and **21l** exhibited improved DPP-IV inhibitory activities ($IC_{50}$ = 0.04-0.059 μM) and compound **21m** showed potent GPR119 agonism (89% activity; $EC_{50}$ = 1.45 μM), were evaluated for their hepatocyte stability and cytotoxicity *in vitro* (**Table 3**). As shown in **Table 3**, the selected compounds with five-member heterocycles displayed good hepatocyte stability in human, but low to medium stability in mouse or rat. The Vero cells were often used in the cytotoxicity experiment *in vitro* to evaluate the preliminary toxicity. Their $IC_{50}$s against Vero cell line were substantially different. Unfortunately, compound **21l** with potent dual activities and relatively good hepatocyte stability exhibited obvious toxicity ($IC_{50}$ = 4.3 μg/mL). Based on the comprehensive analysis of other xanthine compounds, **21h** which possessed favorable human hepatocyte stability and noncytotoxicity was identified as a promising lead for the treatment of diabetes. Indeed, as it was reported

that compound **21h** exerted good activities in ICR mice as well as the diabetic db/db and KKAy mice. Moreover, **21h** can increase first-phase insulin secretion in KKAy mice, suggesting a direct effect on islet β-cells via GPR119 activation [15].

**Table 3.** Hepatocyte stability and cytotoxicity of selected xanthine compounds

| Compds. | Substrate remaining (%)[a] | | | Vero $IC_{50}$ (μg/mL) |
|---|---|---|---|---|
| | Mouse | Rat | Human | |
| **21b** | 21.0 | 19.7 | 68.1 | 25.9 |
| **21d** | 69.4 | 21.7 | 84.0 | 35.2 |
| **21h** | 32.0 | 23.8 | 101.2 | 46.6 |
| **21l** | 68.3 | 39.6 | 91.5 | 4.3 |
| **21m** | 24.2 | 25.0 | 82.0 | 8.7 |

[a]Substrate concentrations were determined in incubations after 30 min and normalized to concentrations at time zero.

3.3 Potential candidate confirmation through salt formation

As shown in **Table 4**, compound **21h** as a free base displayed a relatively low bioavailability (12.2%). It prompted us to improve the bioavailability by forming its hydrochloride **22**. Following a single oral and an intravenous administration of **22**, this compound exhibited high plasma exposure ($AUC_{0-\infty}$ = 656 ng·h/mL), high maximum plasma concentration ($C_{max}$ = 226 ng/ml) and medium elimination half-life ($t_{1/2}$ = 1.08 h) after oral administration dosed at 30 mg/kg (free base content) in rat. The oral bioavailability was increased from 31.8% to 42.4% compared to its free base **21h** at the same dose (30 mg/kg). In addition, compound **22** showed slower $t_{max}$ and longer $MRT_{(0-\infty)}$ in rat after oral administration. These results indicated that compound **22** with superior PK properties may enhance the *in vivo* efficacy in animal models.

**Table 4**. Mouse and Rat Pharmacokinetic Properties of **21h** and **22**

| Parameters | Units | **21h** | | | **22** | |
|---|---|---|---|---|---|---|
| | | Mouse (PO[a]) | Mouse (IV[b]) | Rat (PO) | Rat (PO) | Rat (IV) |
| Dose | mg/kg | 20 | 2 | 30 | 30 | 3 |
| $t_{1/2}$[c] | h | 1.06 | 1.64 | 1.11 | 1.08 | 0.63 |
| $t_{max}$[d] | h | 0.42 | - | 1.17 | 1.33 | - |
| $C_{max}$[e] | ng/mL | 213 | 850 | 192 | 226 | 416 |
| $AUC_{(0-t)}$ | h*ng/mL | 308 | 257 | 468 | 623 | 147 |
| $AUC_{(0-\infty)}$[f] | h*ng/mL | 318 | 261 | 502 | 656 | 167 |

| | | | | | | |
|---|---|---|---|---|---|---|
| $MRT_{(0-\infty)}$[g] | h | 1.22 | 0.76 | 2.11 | 2.22 | 0.73 |
| F[h] | % | 12.2 | | 31.8 | 42.4 | |

[a]Per os. [b]Intravenous injection. [c]Plasma elimination half-life. [d]Time of maximum plasma concentration. [e]Maximum plasma concentration. [f]Plasma exposure. [g]Mean residence time. [h]Bioavailability.

To confirm the pharmacokinetic results of **22** and **21h**, the euglycemic ICR mice was used to explore the glucose-lowering effect (**Fig. 4A**). In ICR mice, a single oral administration of **22** (30 mg/kg, free base content) significantly reduced blood glucose level at 30 minutes after oral glucose loading. Impressively, **22** could achieve a much better *in vivo* effect compared to its free base at the same dose (30 mg/kg) (**Fig. 4A**). The oral glucose tolerance test (OGTT) is the reference method assessing glucose tolerance in the body. It reflects glucose utilization and insulin sensitivity after oral intake of glucose. To further evaluate the anti-diabetic effect of **22**, we performed OGTT in ICR mice with **22** and positive controls (**Fig. 4B**). The area under the curve of OGTT ($AUC_{OGTT}$) of **22**-treated group was significantly decreased by 26.8% at the dose of 30 mg/kg, which was almost equivalent to the AUC of the linagliptin-treated group (26%, 2 mg/kg) and sitagliptin-treated group (27.3%, 10 mg/kg), better than the AUC of APD597-treated group (19.9%, 10 mg/kg) as a positive control of GRP119 agonist (**Fig. 4B**). These findings indicated that compound **22** could achieve comparative glucose-lowering effect *in vivo*. Thus far, all the above results supported compound **22** to be worth of further investigation on druggability.

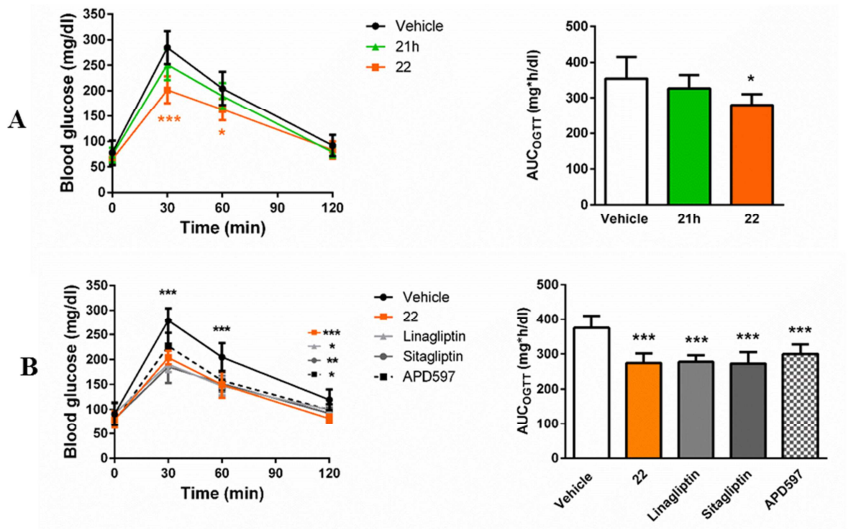



**Fig. 4**. Oral glucose tolerance test in ICR mice. (**A**) Blood glucose level and area under the curve of glucose tolerance test ($AUC_{OGTT}$) measurements after single dose treatment of **21h** and **22**. (**B**)

Blood glucose level and $AUC_{OGTT}$ measurements after single dose treatment of **22** and positive controls. All experiments were performed independently. Data are shown as mean ± SEM of every group, n = 10. *P < 0.05, **P < 0.01, ***P < 0.001, vs vehicle group.

Inspired by the promising PK and PD profiles aforementioned, selected ADME/T studies were conducted on compound **22**. As presented in **Table 5**, compound **22** showed high permeability and moderate metabolic stability. This compound **22** exhibited very high plasma protein binding (PPB, >96%) in five species, possibly due to its high lipophilicity. The ClogP value of **21** (free base form of **22**) is 3.41, while the values of linagliptin and AR231453 are 1.91 and 2.21, respectively, calculated with ChemDraw Professional 16.0.

As depicted in **Table 6**, compound **22** displayed high $IC_{50}$ value against Vero cell, indicating a lack of toxicity to renal cell. A preliminary *in vivo* tolerability study was carried out in mice with a single dose at 1.5 g/kg. All animals survived after oral administration followed by a 7-day observation. Notably, the result of bacterial reverse mutation assay in Salmonella typhimurium tester strains TA98 and TA100 was negative, which suggests low potential for genotoxicity. Maybe attributed its high lipophilicity and aromatic character of linear side chain being curial for GPR119 agonism activity, compound **22** showed moderate inhibition of the hERG channel in a manual patch clamp assay ($IC_{50}$ = 4.9 μM). It precludes that it could be a challenge to balance the physiological properties and biological activities to afford a promising candidate with more potent anti-diabetic activity and lower toxicity targeting DPP-IV and GPR119 at the same time.

**Table 5**. *In vitro* ADME data for compound **22**

| Compd | Papp[a] ($\times10^{-6}$ cm/s) | Mouse Liver Microsome substrate remaining (%)[b] | Plasma Protein Binding (%)[c] | | | | |
|---|---|---|---|---|---|---|---|
| | | | Mouse | Rat | Dog | Monkey | Human |
| **22** | 19.1±1.1 | 49.3% | 98.5 | 96.1 | 96.3 | 96.0 | 97.2 |

[a]Apparent permeability coefficient. [b]Substrate concentrations were determined in incubations with NADPH after 30 min and normalized to concentrations at time zero. [c]Binding at 5 h, starting concentration 5 μM.

**Table 6**. Preliminary toxicity study of compound **22**

| Compd | Cytotoxicity Vero (μg/mL) | Acute Toxicity Study[a] ($LD_{50}$) | Mini-Ames[b] | hERG $K^+$ inhibition $IC_{50}$ (μM) |
|---|---|---|---|---|
| **22** | >64 | > 1.5 g/kg | negative | 4.9 |

[a]No. of animals that survived/total no. of animals after 7 days.

[b]Genetic safety assay using two Salmonella strains: TA98 and TA100.

## 4. Conclusion

In summary, we have synthesized and characterized a series of xanthine compounds derived from our previous hit **20i** modification on the terminal hydrophobic side chain through ring formation strategy. Systematic optimization led to the identification of compound HBK001 hydrochloride **22**, which proved to be a dual modulator targeting DPP-IV and GPR119 with efficient *in vivo* glucose-lowering effect and favorable PK properties. The results of preliminary toxicity investigation exhibited that this compound **22** displayed the moderate inhibition of the hERG channel possibly due to its high lipophilicity. The efforts to maintain the activity but reduce the hERG inhibition through increasing the hydrophilic or the rigidity of the entire molecule are ongoing in order to develop the more promising candidates as anti-diabetic agents with dual mechanism targeting both DPP-IV and GPR119.

## 5. Experimental

### 5.1. Chemistry

#### 5.1.1. General experimental information

Melting points were determined on Yanaco MP-J3 microscope melting point apparatus which is uncorrected. NMR spectra were recorded on Varian-400 and Mercury-500 spectrometer. ESI-HRMS data were measured on Thermo Exactive Orbitrap plus spectrometer. Chemical shifts are referenced to the residual solvent peak and reported in ppm (d scale) and all coupling constant (*J*) values are given in Hz. The following multiplicity abbreviations are used: (s) singlet, (d) doublet, (t) triplet, (q) quartet, (m) multiplet, and (br) broad. TLC was carried out on silica gel plates (GF254) with visualization of components by UV light (254 nm) or exposure to $I_2$.

Column chromatography was carried out on silica gel (200-300 mesh). Petroleum ether (PE), extra pure, boiling range 60-90°C. All the solvents and chemicals were obtained from commercial sources and used without further purification.

### 5.1.2. Preparation of benzyl 4-((2-oxo-2-phenylethyl)carbamoyl)piperidine-1-carboxylate (1e)

1,1'-Carbonyldiimidazole (1.42 g, 8.74 mmol) was added to **1a** (2.00 g, 7.60 mmol) in $CH_2Cl_2$ (15 mL) cooled with an ice bath. The mixture was stirred at room temperature under argon for 3 h. 2-Aminoacetophenone hydrochloride **1d** (1.37 g, 7.98 mmol) and DIPEA (1.39 mL, 7.98 mmol) were then added to the reaction mixture. The mixture was stirred at room temperature for additional 9 h, then evaporated under reduced pressure. The residue was purified by silica gel flash column chromatography ($CH_2Cl_2$/EtOAc = 40:1, V/V) to afford the compound **1e** (1.50 g, 51.9% yield) as a light-brown oil. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 7.97 (m, 2H), 7.63 (m, 1H), 7.51 (m, 2H), 7.34 (m, 5H), 6.60 (brs, 1H), 5.14 (s, 2H), 4.76 (d, *J* = 4.0 Hz, 2H), 4.24 (m, 2H), 2.88 (m, 2H), 2.42 (m, 1H), 1.88 (m, 2H), 1.73 (m, 2H). MS (ESI) m/z 381.19 $[M+H]^+$.

### 5.1.3. Preparation of the piperidyl intermediates 6a-g

#### 5.1.3.1. Preparation of 3-phenyl-5-(piperidin-4-yl)-1,2,4-oxadiazole (6a)

To a solution of **1a** (1.50 g, 5.70 mmol) and DMF (0.03 mL) in anhydrous dichloromethane (5 mL) was added oxalyl chloride (0.68 mL, 7.98 mmol). The mixture was stirred at room temperature for 3 h, then concentrated. The residue was dissolved in toluene (4 mL). A solution of **2** (0.29 g, 3.17 mmol) in toluene (4 mL) was added to the solution at 0 °C, then the mixture was heated to reflux for 4 h. After evaporated under reduced pressure, the residue was purified by silica gel flash column chromatography (PE/EtOAc = 9:1, V/V) to give a yellow oil (0.89 g, 77.4% yield). $^1$H NMR (500 MHz, $CDCl_3$) δ 7.95 (m, 2H), 7.35 (m, 3H), 7.22 (m, 5H), 5.04 (s, 2H), 4.06 (brs, 2H), 2.99 (m, 3H), 1.99 (m, 2H), 1.79 (m, 2H). $^{13}$C NMR (125 MHz,

$CDCl_3$) δ 180.7, 167.9, 154.8, 136.3, 130.8, 128.5, 128.2, 127.7, 127.6, 127.1, 126.5, 66.9, 42.7, 33.9, 28.7.

Iodotrimethylsilane (0.84 mL, 5.88 mmol) was added to the above yellow oil (0.89 g, 2.45 mmol) in $CH_2Cl_2$ (6 mL) cooled with an ice bath. The mixture was stirred at room temperature for 4 h and quenched with methanol (1 mL), followed by the addition of 2N HCl aqueous solution (15mL) and ethyl ether (10 mL). The organic phase was separated and the aqueous phase (15 mL) was basified with $K_2CO_3$ to pH 8-9 and extracted three times with $CH_2Cl_2$ (3×15 mL). The combined organic phases were washed with brine, dried over $Na_2SO_4$, filtered and evaporated *in vacuo* to afford the title compound **6a** (0.49 g, 87.2% yield) as a yellow solid. M.p.: 85-87°C. $^1$H NMR (400 MHz, $CDCl_3$) *δ*: 8.08 (m, 2H), 7.49 (m, 3H), 3.23 (m, 2H), 3.15 (m, 1H), 2.82 (m, 2H), 2.54 (brs, 1H), 2.15 (m, 2H), 1.92 (m, 2H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ 181.9, 168.3, 131.2, 128.9, 127.5, 127.1, 45.7, 34.9, 30.3. MS (ESI) m/z 230.15 $[M+H]^+$.

**5.1.3.2. Preparation of 4-(4-phenyl-1*H*-1,2,3-triazol-1-yl)piperidine (6b)**

To a solution of **1b** (0.23 g, 1.0 mmol), DIPEA (0.65 g, 5.0 mmol) and CuI (0.057 g, 0.03 mmol) in methanol (15 mL) was added phenylacetylene **3** (0.68 mL, 7.98 mmol). The mixture was stirred at room temperature for 2 h, then concentrated. The residue was diluted with EA (50 mL) and $H_2O$ (10 mL). The organic phase was separated and washed with 10% citric acid (10 mL) and brine (10 mL), dried over $Na_2SO_4$, filtered and evaporated *in vacuo* to give a light-yellow solid (0.28 g, 80.3% yield). $^1$H NMR (400 MHz, $CDCl_3$) δ: 7.82 (m, 2H), 7.77 (s, 1H), 7.43 (m, 2H), 7.34 (m, 1H), 4.66 (m, 1H), 4.30 (m, 2H), 2.96 (m, 2H), 2.24 (m, 2H), 2.01 (m, 2H), 1.49 (s, 9H). MS (ESI) m/z 329.25 $[M+H]^+$.

Trifluoroacetic acid (0.7 mL) was added to the above light-yellow solid dissolved in $CH_2Cl_2$ (2 mL). The solution was stirred at room temperature for 2 h. The mixture was diluted with $H_2O$ (15 mL) and ethyl ether (15 mL). The organic phase was separated and the aqueous phase (15 mL) was basified with $K_2CO_3$ to pH 8-9 and

extracted with $CH_2Cl_2$ (5×20 mL). The combined organic phases were washed with brine (10 mL), dried over $Na_2SO_4$, filtered and evaporated *in vacuo* to afford the title compound **6b** (170 mg, 89.0% yield) as an off-white solid. M.p.: 90-92°C. $^1$H NMR (400 MHz, $CDCl_3$) *δ*: 7.83 (m, 2H), 7.78 (s, 1H), 7.43 (m, 2H), 7.32 (m, 1H), 4.62 (m, 1H), 3.28 (m, 2H), 2.82 (m, 2H), 2.24 (m, 2H), 1.99 (m, 2H), 1.57 (brs, 1H). MS (ESI) m/z 229.15 $[M+H]^+$.

**5.1.3.3. Preparation of 4-phenyl-2-(piperidin-4-yl)thiazole (6c)**

To a solution of **1c** (0.30 g, 1.23 mmol) and $NaHCO_3$ (0.21 g, 2.46 mmol) in ethanol (5 mL) was added α-bromoacetophenone (0.26 g, 1.29 mmol). The mixture was heated to reflux for 3 h. After evaporated under reduced pressure, the residue was purified by silica gel flash column chromatography ($CH_2Cl_2$/EtOAc = 40:1, V/V) to give a light-brown oil (0.40 g, 93.2% yield). $^1$H NMR (400 MHz, $CDCl_3$) δ: 7.90 (m, 2H), 7.42 (m, 2H), 7.38 (s, 1H), 7.34 (m, 1H), 4.21 (m, 2H), 3.33 (m, 1H), 2.93 (m, 2H), 2.17 (m, 2H), 1.77 (m, 2H), 1.48 (s, 9H). MS (ESI) m/z 345.18 $[M+H]^+$.

Trifluoroacetic acid (1.0 mL) was added to the above light-brown oil (0.39 g, 1.15 mmol) dissolved in $CH_2Cl_2$ (3 mL). The solution was stirred at room temperature for 2 h. The mixture was diluted with $H_2O$ (15 mL) and ethyl ether (10 mL). The organic phase was separated and the aqueous phase (15 mL) was basified with $K_2CO_3$ to pH 8-9 and extracted with $CH_2Cl_2$ (3×20 mL). The combined organic phases were washed with brine, dried over $Na_2SO_4$, filtered and evaporated *in vacuo* to afford the title compound **6c** (0.24 g, 85.7% yield) as an off-white solid. M.p.: 76-78°C. $^1$H NMR (400 MHz, $CDCl_3$) *δ*: 7.88 (m, 2H), 7.41 (m, 2H), 7.35 (s, 1H), 7.30 (m, 1H), 3.20 (m, 3H), 2.80 (m, 2H), 2.17 (m, 2H), 1.73-1.84 (m, 3H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ 175.8, 155.0, 134.9, 128.8, 128.1, 126.5, 111.7, 46.5, 41.3, 34.0. MS (ESI) m/z 245.13 $[M+H]^+$.

**5.1.3.4. Preparation of 4-(4-phenyl-1*H*-imidazol-2-yl)piperidine (6d)**

To a solution of **1e** (0.73 g, 1.92 mmol) in acetic acid (5 mL) was added

ammonium acetate (2.07 g, 26.86 mmol). The mixture was heated to reflux for 5 h. After cooling to room temperature, the reaction mixture was poured into water (20 mL) and extracted with $CH_2Cl_2$ (3×20 mL). The combined organic phases were washed with brine, dried over $Na_2SO_4$, then filtered. After evaporated under reduced pressure, the residue was purified by silica gel flash column chromatography (PE/EtOAc = 1:1, V/V) to give a light-yellow oil (0.66 g, 83.7% yield). $^1$H NMR (400 MHz, $CDCl_3$) δ: 7.67 (m, 2H), 7.38 (m, 7H), 7.23 (m, 2H), 5.13 (s, 2H), 4.28 (m, 2H), 3.04 (m, 1H), 2.93 (m, 2H), 2.05 (m, 2H), 1.73 (m, 2H).

Iodotrimethylsilane (0.60 mL, 4.15 mmol) was added to the above light-yellow oil (0.63 g, 1.73 mmol) in anhydrous dichloromethane (10 mL) at room temperature under argon. The mixture was stirred for 4 h and quenched with methanol (1 mL), followed by the addition of 2N HCl aqueous solution (15mL) and ethyl ether (10 mL). The organic phase was separated and the aqueous phase (15 mL) was basified with $K_2CO_3$ to pH 8-9 and extracted with $CH_2Cl_2$ (3×15 mL). The combined organic phases were washed with brine, dried over $Na_2SO_4$, filtered and evaporated *in vacuo* to afford the title compound **6d** (0.44 g, 99.3% yield) as an off-white solid. M.p.: 92-94°C. $^1$H NMR (400 MHz, $CDCl_3$) *δ*: 7.68 (m, 2H), 7.35 (m, 2H), 7.21 (m, 2H), 3.18 (m, 2H), 2.94 (m, 1H), 2.72 (m, 2H), 2.02 (m, 2H), 1.76 (m, 2H). MS (ESI) m/z 228.97 $[M+H]^+$.

**5.1.3.5. Preparation of 5-phenyl-2-(piperidin-4-yl)oxazole (6e)**

To a solution of **1e** (0.80 g, 2.10 mmol) in pyridine (5 mL) was added phosphorus oxychloride (1.0 mL, 10.5 mmol). The mixture was stirred at room temperature for 2 h and then slowly poured into saturated $NaHCO_3$ solution (20 mL) cooled by ice bath. The aqueous phase was extracted with ethyl acetate (3×20 mL) and the combined organic phases were dried over $Na_2SO_4$, filtered and evaporated *in vacuo*. The resulting residue was purified by silica gel flash column chromatography (PE/EtOAc = 1:1, V/V) to give a light-yellow oil (0.66 g, 86.8% yield). $^1$H NMR (400 MHz, $CDCl_3$) δ: 7.60 (m, 2H), 7.37 (m, 8H), 7.24 (s, 1H), 5.15 (s, 2H), 4.20 (m, 2H), 3.07

(m, 2H), 2.11 (m, 2H), 1.05 (m, 3H). MS (ESI) m/z 363.17 $[M+H]^+$ .

Iodotrimethylsilane (0.66 mL, 4.64 mmol) was added to the above light-yellow oil (0.60 g, 1.93 mmol) in anhydrous dichloromethane (10 mL) at room temperature under argon. The mixture was stirred for 4 h and quenched with methanol (1 mL), followed by the addition of 2N HCl aqueous solution (15mL) and ethyl ether (10 mL). The organic phase was separated and the aqueous phase (15 mL) was basified with $K_2CO_3$ to pH 8-9 and extracted with $CH_2Cl_2$ (3×15 mL). The combined organic phases were washed with brine (15 mL), dried over $Na_2SO_4$, filtered and evaporated *in vacuo* to afford the title compound **6e** (0.43 g, 96.4% yield) as an off-white solid. M.p.: 62-64°C. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 7.61 (m, 2H), 7.40 (m, 2H), 7.30 (m, 1H), 7.22 (s, 1H), 3.19 (m, 2H), 3.00 (m, 1H), 2.77 (m, 2H), 2.10 (m, 2H), 1.96 (brs, 1H), 1.84 (m, 2H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ 166.7, 150.9, 129.0, 128.3, 128.3, 124.1, 121.7, 46.0, 36.2, 30.8. MS (ESI) m/z 229.13 $[M+H]^+$.

**5.1.3.6. Preparation of 5-phenyl-3-(piperidin-4-yl)isoxazole (6f)**

To a solution of **1f** (2.13 g, 9.33 mmol), phenylacetylene **3** (0.68 mL, 7.98 mmol) and KCl (0.70 g, 9.33 mmol) in water (20 mL) was added $HKO_6S$ (8.57 g, 13.95 mmol). The mixture was stirred at room temperature overnight and then extracted with $CH_2Cl_2$ (3×30 mL). The combined organic phases were washed with brine, dried over $Na_2SO_4$, filtered and evaporated under reduce pressure. The resulting residue was purified by silica gel flash column chromatography ($CH_2Cl_2$/EtOAc = 40:1, V/V) to give a colorless oil (1.16 g, 37.9% yield). $^1$H NMR (400 MHz, $CDCl_3$) δ: 7.76 (m, 2H), 7.46 (m, 3H), 6.38 (s, 1H), 4.18 (m, 2H), 2.94 (m, 3H), 1.98 (m, 2H), 1.72 (m, 2H), 1.48 (s, 9H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ 170.0, 167.5, 154.9, 130.2, 129.1, 127.7, 125.9, 97.7, 79.8, 43.7, 34.4, 31.0, 28.6. MS (ESI) m/z 351.17 $[M+H]^+$.

Trifluoroacetic acid (3.0 mL) was added to the above colorless oil (1.10 g, 3.55 mmol) dissolved in $CH_2Cl_2$ (10 mL). The solution was stirred at room temperature for 2 h. The mixture was diluted with $H_2O$ (20 mL) and ethyl ether (20 mL). The organic phase was separated and the aqueous phase (20 mL) was basified with $K_2CO_3$ to pH

8-9 and extracted with $CH_2Cl_2$ (3×30 mL). The combined organic phases were washed with brine (20 mL), dried over $Na_2SO_4$, filtered and evaporated *in vacuo* to afford the title compound **6f** (0.65 g, 85.0% yield) as an off-white solid. M.p.: 96-99°C.$^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 7.82 (m, 2H), 7.50 (m, 3H), 6.98 (s, 1H), 3.83 (brs, 1H), 3.05 (m, 2H), 2.86 (m, 1H), 2.66 (m, 2H), 1.87 (m, 2H), 1.59 (m, 2H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ 169.9, 168.0, 130.2, 129.1, 127.7, 125.9, 97.7, 46.2, 34.4, 31.9. MS (ESI) m/z 229.13 $[M+H]^+$.

**5.1.3.7. Preparation of 4-(1-phenyl-1*H*-tetrazol-5-yl)piperidine hydrochloride (6g)**

To a solution of **1g** (1.00 g, 4.37 mmol) in acetonitrile (10 mL) was added EDCI (0.84 g, 44 mmol), HOBt (0.59 g, 4.37 mmol), triethylamine (0.68 mL, 4.81 mmol) and aniline **5** (0.44 g, 4.37 mmol) in turn. The mixture was stirred at room temperature for 5 h and then diluted with $CH_2Cl_2$ (50 mL) and saturated $NaHCO_3$ solution (10 mL). The organic phase was separated and washed with brine (20 mL), dried over $Na_2SO_4$, filtered and evaporated *in vacuo* to give an off-white solid (0.83 g, 62.5% yield). $^1$H NMR (400 MHz, $CDCl_3$) δ 7.52 (m, 3H), 7.31 (t, $J$ = 7.9 Hz, 2H), 7.10 (t, $J$ = 7.4 Hz, 1H), 4.18 (m, 2H), 2.76 (m, 2H), 2.38 (m, 1H), 1.87 (m, 2H), 1.75 (m, 2H), 1.47 (s, 9H).

To a solution of the above off-white solid (0.40 g, 1.32 mmol), triphenylphosphine (0.80 g, 2.63 mmol) and TMS-$N_3$ (0.35 mL, 2.63 mmol) in anhydrous THF (10 mL), DIAD (0.52 mL, 2.63 mmol) was added dropwise cooled with an ice bath. The mixture was stirred at room temperature for 6 days, and then heated to reflux for 1 h. After cooling to room temperature, 7 N HCl-EA solution (10 mL) was added to the mixture and stirred at room temperature for additional 1 h. The mixture was diluted with ethyl acetate (10 mL) and then filtered. The residue was washed with ethyl acetate to afford the title compound **6g** (0.30 g, 86.0%) as an off-white solid. M.p.:110-112°C. $^1$H NMR (400 MHz, DMSO-$d_6$) δ: 9.29 (brs, 1H), 9.16 (brs, 1H), 8.35 (brs, 1H), 7.68 (m, 5H), 3.29 (m, 3H), 2.96 (m, 2H), 2.02 (m, 4H).

MS (ESI) m/z 230.14 $[M+H]^+$.

### 5.1.4. Preparation of 3-phenyl-5-(trichloromethyl)-1,2,4-oxadiazole (7)

To a solution of **2** (2.50 g, 18.36 mmol) in toluene (20 mL) was added trichloroacetic anhydride (4.30 mL, 23.50 mmol) dropwise and the mixture was heated to reflux for 10 h. After evaporated under reduced pressure, the residue was diluted with ethyl acetate (30 mL). The organic phase was washed with brine (15 mL), dried over $Na_2SO_4$, filtered and evaporated *in vacuo*. The residue was purified by silica gel flash column chromatography (PE/$CH_2Cl_2$ = 100:1) to give a colorless oil (4.39 g, 90.7% yield). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 8.13 (m, 2H), 7.53 (m, 3H).

### 5.1.5. 5-Chloro-3-phenyl-1,2,4-thiadiazole (9)

To a solution of **8** (0.50 g, 3.19 mmol) and (trichloromethyl)sulfenyl chloride (10 mL) in $CH_2Cl_2$ (10 mL) cooled by ice bath was added NaOH (0.64 g, 15.96 mmol) and the mixture was stirred for 1 h, then stirred at room temperature for additional 2 h. The mixture was diluted with $CH_2Cl_2$ (30 mL). The combined organic phases were washed with brine, dried over $Na_2SO_4$, filtered and evaporated *in vacuo* to give a light-yellow oil. The product was used to the next step without further purification.

### 5.1.6. Preparation of *tert*-butyl 4-(benzoylcarbamothioyl)piperazine-1-carboxylate (11)

To a solution of 1-Boc-piperazine (0.50 g, 2.68 mmol) in anhydrous $CH_2Cl_2$ (10 mL), a solution of **10** (0.38 mL, 2.68 mmol) in $CH_2Cl_2$ (5 mL) was added dropwise cooled by ice bath. The mixture was stirred at room temperature for 2 h. After evaporated under reduced pressure, the resulting residue was washed with the mixed solvent (6 mL, diethyl ether/hexane = 1:2, V/V) to give an off-white solid (0.85 g, 90.6%). M.p.: 142-145 °C. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 8.49 (brs, 1H), 7.83 (m, 2H), 7.60 (m, 1H), 7.50 (m, 2H), 4.18 (m, 2H), 3.64 (m, 6H), 1.48 (s, 9H). MS (ESI) m/z 372.13 $[M+Na]^+$.

**5.1.7. Preparation of *tert*-butyl 4-carbamothioylpiperazine-1-carboxylate (14)**

Intermediate **11** (0.66 g, 1.86 mmol) was dissolved in 50% hydrazine hydrate aqueous solution (3 mL). The mixture was stirred at room temperature for 4 h, then diluted with $CH_2Cl_2$ (30 mL). The organic phase was washed with 10% citric acid (2×30 mL) and brine (25 mL), dried over $Na_2SO_4$, filtered and evaporated under reduced pressure. The resulting residue was washed with diethyl ether (20 mL) to give an off-white solid (0.38 g, 81.1% yield). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 5.82 (brs, 2H), 3.84 (m, 4H), 3.55 (m, 4H), 1.47 (s, 9H). MS (ESI) m/z 246.13 $[M+H]^+$.

**5.1.8. Preparation of *tert*-butyl (*Z*)-4-(*N'*-hydroxycarbamimidoyl)piperazine-1-carboxylate (15)**

To a solution of 1-Boc-piperazine **13** (0.50 g, 2.68 mmol) and $NaHCO_3$ aqueous solution (1.10 g in 20 mL $H_2O$) in $CH_2Cl_2$ (10mL), a solution of cyanogen bromide (0.34 g, 3.22 mmol) in $CH_2Cl_2$ (10 mL) was added dropwise cooled by ice bath. The mixture was stirred at room temperature overnight, then diluted with the saturated $NaHCO_3$ solution (10 mL) and extracted with $CH_2Cl_2$ (3×15 mL). The combined organic phases were washed with brine (20 mL), dried over $Na_2SO_4$, filtered and evaporated *in vacuo* to afford a white solid (0.54 g, 95.4% yield). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 3.53-3.50 (m, 4H), 3.20-3.18 (m, 4H), 1.46 (s, 9H).

50% hydroxylamine aqueous solution (0.15 mL, 2.45 mmol) was added to the solution of the above white solid (0.50 g, 2.37 mmol) in ethanol (5 mL). The mixture was heated to reflux for 6 h, then concentrated. The residue was dissolved in toluene (10 mL) and evaporated *in vacuo* to afford an off-white solid (0.53 g, 91.5% yield). M.p.: 164-166 °C. $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 4.50 (s, 1H), 3.57-3.43 (m, 4H), 3.13 (m, 3H), 3.00-2.93 (m, 1H), 1.55-1.47 (m, 9H).

**5.1.9. Preparation of the piperazinyl intermediates 16a-f**

**5.1.9.1. Preparation of 3-phenyl-5-(piperazin-1-yl)-1,2,4-oxadiazole (16a)**

A solution of anhydrous piperazine (2.8 g, 33.32 mmol) in DMF (20 mL) was added slowly to the mixture of **7** (4.39 g, 16.66 mmol) and DMF (15 mL). The mixture was stirred at room temperature for 6 h. After evaporated under reduced pressure, the residue was diluted with EA (200 mL) and washed with brine (100 mL×2), dried over $Na_2SO_4$, filtered and evaporated under reduced pressure. The residue was purified by silica gel flash column chromatography ($CH_2Cl_2$/MeOH = 100:1, V/V) to afford the title compound **16a** (2.35 g, 61.2% yield) as a light-yellow solid. M.p.: 65-67°C. $^1$H NMR (400 MHz, $CDCl_3$) *δ*: 7.99 (m, 2H), 7.44 (m, 3H), 3.73 (m, 4H), 3.04 (m, 4H), 2.16 (brs, 1H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ 171.2, 168.8, 130.8, 128.7, 127.9, 127.3, 47.0, 45.5. MS (ESI) m/z 231.13 $[M+H]^+$.

**5.1.9.2. Preparation of 3-phenyl-5-(piperazin-1-yl)-1,2,4-thiadiazole (16b)**

Triethylamine (1.8 mL, 12.77 mmol) was added to a solution of **9** and 1-Boc-piperazine (0.60 g, 3.19 mmol) in DMF (4 mL). The mixture was stirred at room temperature overnight, then concentrated. The residue was diluted with water (10 mL) and extracted with ethyl acetate (3×20 mL). The combined organic phases were washed with brine (20 mL), dried over $Na_2SO_4$, filtered. After evaporated under reduced pressure, the residue was purified by silica gel flash column chromatography (PE/ EtOAc = 10:1, V/V) to give a light-yellow solid (0.62 g, 55.6% yield over two steps). $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.19 (m, 2H), 7.42 (m, 3H), 3.60 (m, 8H), 1.49 (s, 9H). MS (ESI) m/z 347.17 $[M+H]^+$.

Trifluoroacetic acid (5.0 mL) was added to the above light-yellow solid (0.61 g, 1.76 mmol) dissolved in $CH_2Cl_2$ (5 mL). The solution was stirred at room temperature for 2 h. The mixture was diluted with $H_2O$ (15 mL) and ethyl ether (15 mL). The organic phase was separated and the aqueous phase (15 mL) was basified with $K_2CO_3$ to pH 8-9 and extracted with $CH_2Cl_2$ (4×20 mL). The combined organic phases were washed with brine, dried over $Na_2SO_4$, filtered and evaporated *in vacuo* to afford the title compound **16b** (0.34 g, 79.2% yield) as an off-white solid. M.p.:78-79°C. $^1$H NMR (400 MHz, $CDCl_3$) *δ*: 8.19 (m, 2H), 7.42 (m, 3H), 3.60 (m, 4H), 3.03 (m, 4H),

2.08 (brs, 1H). MS (ESI) m/z 247.11 $[M+H]^+$.

**5.1.9.3. Preparation of 1-(5-phenyl-4*H*-1,2,4-triazol-3-yl)piperazine (16c)**

Hydrazine monohydrate (Hydrazine, 64%) (0.59 g, 12 mmol) was added to a solution of **11** (0.85 g, 2.3 mmol) in chloroform (20 mL). The mixture was heated to reflux for 4 h, then concentrated. The residue was diluted with water (30 mL) and extracted with ethyl acetate (3×30 mL). The combined organic phases were washed with brine, dried over $Na_2SO_4$, filtered. After evaporated under reduced pressure, the residue was purified by silica gel flash column chromatography (PE/ EtOAc = 2:1, V/V) to give an off-white solid (0.46 g, 61.3% yield). M.p.:176-178 °C. $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.81 (brs, 1H), 7.89 (m, 2H), 7.41 (m, 3H), 3.52 (m, 4H), 3.45 (m, 4H), 1.48 (s, 9H). $^{13}$C NMR (125 MHz, $CDCl_3$) δ 163.0, 157.5, 154.8, 129.8, 129.2, 128.8, 126.3, 80.2, 46.5, 43.1, 28.5. MS (ESI) m/z 330.24 $[M+H]^+$.

Trifluoroacetic acid (2.0 mL) was added to the above off-white solid (0.46 g, 1.40 mmol) dissolved in $CH_2Cl_2$ (5 mL). The solution was stirred at room temperature for 2 h. The mixture was diluted with $H_2O$ (15 mL) and ethyl ether (15 mL). The organic phase was separated and the aqueous phase (15 mL) was basified with $K_2CO_3$ to pH 8-9 and extracted with $CH_2Cl_2$ (4×30 mL). The combined organic phases were washed with brine, dried over $Na_2SO_4$, filtered and evaporated *in vacuo* to afford the title compound **16c** (0.26 g, 81.3% yield) as an off-white solid. M.p.: 160-161°C. $^1$H NMR (400 MHz, $CDCl_3$) *δ*: 7.91 (m, 2H), 7.39 (m, 3H), 5.14 (brs, 1H), 3.42 (m, 4H), 2.96 (m, 4H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ 162.6, 158.3, 129.9, 129.7, 128.8, 126.4, 47.6, 45.3. MS (ESI) m/z 230.14 $[M+H]^+$.

**5.1.9.4. Preparation of 1-(3-methyl-1-phenyl-1*H*-pyrazol-5-yl)piperazine (16d)**

**12** (0.95 g, 6.40 mmol) was added to a solution of 1-Boc-piperazine (1.43 g, 7.68 mmol) in anhydrous ethanol (30 mL). The mixture was heated to reflux for 3 h, followed by addition of phenylhydrazine (0.75 mL, 7.68 mmol), acetic acid (5 drops) and 4Å molecular sieve (1.28 g). The mixture was heated to reflux for additional 12 h,

then concentrated. The mixture was diluted with $CH_2Cl_2$ (90 mL), washed subsequently with 1 mol/L HCl (10 mL), water (10 mL), saturated $NaHCO_3$ solution (10 mL) and brine (10 mL), dried over $Na_2SO_4$, filtered and evaporated *in vacuo*. The residue was purified by silica gel flash column chromatography (PE/ EtOAc = 9:1, V/V) to give a white solid (1.77 g, 80.7% yield). M.p.: 92-95°C. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 7.77 (m, 2H), 7.40 (m, 2H), 7.26 (m, 1H), 5.68 (s, 1H), 3.45 (m, 4H), 2.81 (brs, 4H), 2.27 (s, 3H), 1.45 (s, 9H).

Trifluoroacetic acid (4 mL) was added to the above white solid (1.14 g, 3.33 mmol) dissolved in $CH_2Cl_2$ (12 mL). The solution was stirred at room temperature for 1.5 h. The mixture was diluted with $H_2O$ (15 mL) and ethyl ether (15 mL). The organic phase was separated and the aqueous phase (15 mL) was basified with $K_2CO_3$ to pH 8-9 and extracted with ethyl acetate (3×40 mL). The combined organic phases were washed with brine (15 mL), dried over $Na_2SO_4$, filtered and evaporated *in vacuo* to afford the title compound **16d** (0.79 g, 97.8% yield) as an off-white solid. M.p.: 103-105°C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 7.76 (d, $J$ = 8.0 Hz, 2H), 7.45 (t, $J$ = 8.0 Hz, 2H), 7.26 (t, $J$ = 7.6 Hz, 1H), 5.77 (s, 1H), 2.72 (brs, 4H), 2.70 (brs, 4H), 2.50 (s, 3H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ 152.7, 149.0, 140.4, 129.0, 126.4, 122.8, 94.2, 52.3, 45.7, 14.2. MS (ESI) m/z 243.17 $[M+H]^+$.

**5.1.9.5. Preparation of 4-phenyl-2-(piperazin-1-yl)thiazole (16e)**

To a solution of **14** (0.38 g, 1.55 mmol), $NaHCO_3$ (0.26 g, 3.10 mmol) in ethanol (5 mL) was added 2-bromoacetophenone (0.33 g, 1.64 mmol). The mixture was heated to reflux for 4 h, then evaporated *in vacuo*. The residue was purified by silica gel flash column chromatography (PE/ $CH_2Cl_2$ = 1:1, V/V) to give a light-yellow oil (0.43 g, 80.0% yield). $^1$H NMR (400 MHz, $CDCl_3$) δ: 7.83 (m, 2H), 7.39 (m, 2H), 7.30 (m, 1H), 6.78 (s, 1H), 3.59 (m, 8H), 1.49 (s, 9H).

Trifluoroacetic acid (1 mL) was added to the above light-yellow oil (0.38 g, 1.10 mmol) dissolved in $CH_2Cl_2$ (3 mL). The solution was stirred at room temperature for 2 h. The mixture was diluted with $H_2O$ (10 mL) and ethyl ether (10 mL). The organic

phase was separated and the aqueous phase (10 mL) was basified with $K_2CO_3$ to pH 8-9 and extracted with $CH_2Cl_2$ (3×15 mL). The combined organic phases were washed with brine (15 mL), dried over $Na_2SO_4$, filtered and evaporated *in vacuo* to afford the title compound **16e** (0.24 g, 87.4% yield) as a light-yellow oil. $^1$H NMR (400 MHz, $CDCl_3$) *δ*: 7.82 (m, 2H), 7.37 (m, 2H), 7.28 (m, 1H), 6.80 (s, 1H), 3.64 (m, 4H), 3.11 (m, 4H). MS (ESI) m/z 246.13 $[M+H]^+$.

**5.1.9.6. Preparation of 5-phenyl-3-(piperazin-1-yl)-1,2,4-oxadiazole (16f)**

To a solution of benzoic acid (0.50 g, 4.09 mmol) and **15** (0.83 g, 3.41 mmol) in anhydrous DMF (5 mL) was added CDI (0.66 g, 4.09 mmol). The mixture was stirred at room temperature for 3 h, followed by the addition of CDI (0.66 g, 4.09 mmol). The reaction mixture was heated to 120°C for additional 8 h. The reaction was quenched by water (15mL) and extracted with ethyl acetate (3×15 mL). The organic layer was washed with brine (20 mL) and dried over $Na_2SO_4$, filtered and evaporated *in vacuo*. The residue was purified by silica gel flash column chromatography ($CH_2Cl_2$/$CH_3OH$ = 50:1, V/V) to give a white solid (0.84 g, 62.2% yield). M.p.: 92-95°C. $^1$H NMR (500 MHz, $CDCl_3$) δ 8.08-8.06 (m, 2H), 7.58-7.48 (m, 3H), 3.56-3.55 (m, 4H), 3.52 (brs, 4H), 1.49 (s, 9H).

Trifluoroacetic acid (1 mL) was added to the above white solid (0.20 g, 0.61 mmol) dissolved in $CH_2Cl_2$ (3 mL). The solution was stirred at room temperature for 2 h. The mixture was diluted with $H_2O$ (10 mL) and ethyl ether (10 mL). The organic phase was separated and the aqueous phase (10 mL) was basified with $K_2CO_3$ to pH 8-9 and extracted with $CH_2Cl_2$ (3×15 mL). The combined organic phases were washed with brine, dried over $Na_2SO_4$, filtered and evaporated *in vacuo* to afford the title compound **16f** (0.11 g, 79.7% yield) as a light-yellow oil. $^1$H NMR (400 MHz, $CDCl_3$) δ 8.08-8.06 (m, 2H), 7.58-7.48 (m, 3H), 3.56-3.53 (m, 4H), 3.03-3.01 (m, 4H), 2.23 (brs, 1H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ 174.5, 171.0, 132.5, 129.0, 128.0, 124.9, 47.2, 45.5. MS (ESI): m/z 231.12 $[M+H]^+$

**5.1.10. General procedure for preparation of intermediate pyrimidinyl 19a-f**

To a solution of **17a-f** (7.78 mmol), 1-Boc-piperazine **13** (7.78 mmol) in anhydrous ethanol (10 mL) was added triethylamine (15.56 mmol). The mixture was heated to reflux for 12 h. After cooling to the ambient temperature, ice-cold water (20 mL) was added. The precipitate was separated by filtration, washed with water (10 mL) and dried to afford **18a-f**. The compounds **18a-f** were used to the next step without further purification.

Trifluoroacetic acid (1 mL) was added to **18a-f** (3.60 mmol) dissolved in $CH_2Cl_2$ (3 mL). The solution was stirred at room temperature for 2 h. The mixture was diluted with $H_2O$ (10 mL) and ethyl ether (10 mL). The organic phase was separated and the aqueous phase (10 mL) was basified with $K_2CO_3$ to pH 8-9 and extracted with $CH_2Cl_2$ (3×15 mL). The combined organic phases were washed with brine, dried over $Na_2SO_4$, filtered and evaporated *in vacuo* to afford the title compounds **19a-f** below.

**5.1.10.1. Preparation of 5-methyl-2-(piperazin-1-yl)pyrimidine (19a).** Yield: 77.2%. Light-yellow oil. $^1$H NMR (400 MHz, $CDCl_3$) δ 8.16 (s, 2H), 3.80 (t, *J* = 4.8 Hz, 4H), 2.97 (t, *J* = 5.2 Hz, 4H), 2.12 (s, 3H). MS (ESI): m/z 179.13 $[M+H]^+$.

**5.1.10.2. Preparation of 5-ethyl-2-(piperazin-1-yl)pyrimidine (19b).** Yield: 53.0%. Colorless oil. $^1$H NMR (400 MHz, $CDCl_3$) δ 8.17 (s, 2H), 3.77-3.74 (m, 4H), 2.95-2.92 (m, 4H), 2.46 (q, *J* = 7.6 Hz, 2H), 2.12 (s, 1H), 1.20-1.16 (m, 3H). MS (ESI): m/z 193.14 $[M+H]^+$.

**5.1.10.3. Preparation of 2-(piperazin-1-yl)-5-propylpyrimidine (19c).** Yield: 68.7%. Light-yellow oil. $^1$H NMR (500 MHz, $CDCl_3$) δ 8.15 (s, 2H), 3.76 (brs, 4H), 2.94 (brs, 4H), 2.39 (t, *J* = 7.5 Hz, 2H), 2.11 (brs, 1H), 1.58-1.54 (m, 2H), 0.93 (t, *J* = 7.5 Hz, 3H). MS (ESI): m/z 207.17 $[M+H]^+$.

**5.1.10.4. Preparation of 5-methoxy-2-(piperazin-1-yl)pyrimidine (19d).** Yield:

53.8%. Light-yellow oil. $^1$H NMR (500 MHz, $CDCl_3$) δ 8.09 (s, 2H), 3.79 (s, 3H), 3.72-3.70 (m, 4H), 2.96-2.94 (m, 4H), 2.24 (s, 1H). MS (ESI): m/z 195.15 $[M+H]^+$.

**5.1.10.5. Preparation of 5-fluoro-2-(piperazin-1-yl)pyrimidine (19e).** Yield: 77.4%. Yellow oil. $^1$H NMR (400 MHz, $CDCl_3$) δ 8.20 (s, 2H), 3.78-3.76 (m, 4H), 2.97-2.95 (m, 4H), 2.75 (brs, 1H). MS (ESI): m/z 183.11 $[M+H]^+$.

**5.1.10.6. Preparation of 2-(piperazin-1-yl)-5-(trifluoromethyl)pyrimidine (19f).** Yield: 39.6%. Colorless oil. $^1$H NMR (400 MHz, $CDCl_3$) δ 8.49 (s, 2H), 3.94 (t, *J* = 5.4 Hz, 4H), 3.48 (brs, 1H), 2.98 (t, *J* = 5.4 Hz, 4H).

**5.1.11. General procedure for preparation of the target compounds 21a-s**

To a solution of **6a-g**, **16a-f** or **19a-f** (0.35 mmol) in DMF (3 mL) was added **20** (0.34 mmol) (prepared according to the method reported by our previous work [10]) and DIPEA (1.02 mmol). The mixture was heated at 80°C under argon for 10 h, then concentrated. The residue was diluted with ethyl acetate (25 mL), washed with brine (25 mL), dried over $Na_2SO_4$, filtered and evaporated *in vacuo* to give an oil or a solid which was used to the next step without further purification. Trifluoroacetic acid (1 mL) was added to the *N*-Boc protected oil or solid (0.34 mmol) dissolved in $CH_2Cl_2$ (3 mL). The solution was stirred at room temperature for 2 h and then poured into ice-cold water (15 mL). The organic phase was separated and the aqueous phase (15 mL) was basified with $K_2CO_3$ and extracted with $CH_2Cl_2$ (3×15 mL). The combined organic phases were dried over $Na_2SO_4$, filtered and evaporated *in vacuo*. The residue was purified by silica gel flash column chromatography ($CH_2Cl_2$/MeOH = 20:1, V/V) to afford the title compounds **21a-s** below.

**5.1.11.1. Preparation of (*R*)-8-(3-aminopiperidin-1-yl)-7-(but-2-yn-1-yl)-3-methyl-1-(3-(4-(3-phenyl-1,2,4-oxadiazol-5-yl)piperidin-1-yl)propyl)-1*H*-purine-2,6(3*H*,7*H*)-dione (21a).**

Compound **21a** was synthesized from compounds **6a** and **20** according to the general method. Yield: 63.4%. Light-brown solid. M.p.: 100-102°C. $[\alpha]_D^{20}$ -12.42 (*c* 1.03, $CHCl_3$). $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$: 8.06 (m, 2H), 7.48 (m, 3H), 4.86 (m, 2H), 4.07 (t, *J* = 7.2 Hz, 2H), 3.62 (m, 1H), 3.49 (s, 3H), 3.47 (m, 1H), 3.05 (m, 5H), 2.87 (m, 1H), 2.55 (m, 2H), 2.15 (m, 4H), 1.69-2.08 (m, 12H), 1.36 (m, 1H). $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta$: 181.6, 168.3, 155.8, 154.5, 151.7, 147.6, 131.3, 129.0, 127.6, 127.0, 104.6, 81.6, 73.3, 58.6, 56.1, 55.8, 52.3, 50.9, 47.2, 39.7, 35.7, 29.8, 28.8, 24.8, 22.6, 18.6, 3.9. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{31}H_{40}N_9O_3$: 586.32486; found: 586.32300.

**5.1.11.2. Preparation of (*R*)-8-(3-aminopiperidin-1-yl)-7-(but-2-yn-1-yl)-3-methyl-1-(3-(4-(4-phenyl-1*H*-1,2,3-triazol-1-yl)piperidin-1-yl)propyl)-1*H*-purine-2,6(3*H*,7*H*)-dione (21b).** Compound **21b** was synthesized from compounds **6b** and **20** according to the general method. Yield: 64.2%. Off-white solid. M.p.: 95-97°C. $[\alpha]_D^{20}$ -17.16 (*c* 1.04, $CHCl_3$). $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$: 7.82 (m, 2H), 7.75 (s, 1H), 7.43 (m, 2H), 7.33 (m, 1H), 4.86 (m, 2H), 4.11 (t, *J* = 7.2 Hz, 2H), 3.59 (m, 1H), 3.53 (s, 3H), 3.48 (m, 1H), 3.06 (m, 4H), 2.86 (m, 1H), 2.53 (m, 2H), 2.14 (m, 4H), 1.90 (m, 5H), 1.81 (s, 3H), 1.69 (m, 1H), 1.65 (brs, 2H), 1.31 (m, 1H). $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta$: 156.1, 154.6, 151.7, 147.6, 130.8, 129.0, 128.2, 125.8, 117.3, 104.7, 81.4, 73.3, 58.5, 57.7, 56.0, 52.3, 52.2, 50.6, 47.3, 39.9, 35.7, 32.9, 29.7, 25.0, 23.1, 3.8. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{31}H_{41}N_{10}O_2$: 585.34085; found: 585.33856.

**5.1.11.3. Preparation of (*R*)-8-(3-aminopiperidin-1-yl)-7-(but-2-yn-1-yl)-3-methyl-1-(3-(4-(4-phenylthiazol-2-yl)piperidin-1-yl)propyl)-1*H*-purine-2,6(3*H*,7*H*)-dione (21c).** Compound **21c** was synthesized from compounds **6c** and **20** according to the general method. Yield: 66.2%. Off-white solid. M.p.: 78-80°C. $[\alpha]_D^{20}$ -16.49 (*c* 0.99, $CHCl_3$). $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$: 7.87 (m, 2H), 7.40 (m, 2H), 7.31 (m, 2H), 4.85 (m, 2H), 4.07 (t, *J* =

7.2 Hz, 2H), 3.61 (m, 1H), 3.54 (m, 1H), 3.51 (s, 3H), 3.05 (m, 5H), 2.84 (m, 1H), 2.52 (m, 2H), 2.15 (m, 4H), 1.69-2.16 (m, 12H), 1.32 (m, 1H). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$: 175.6, 156.2, 154.9, 151.7, 147.7, 134.8, 128.8, 128.0, 126.5, 111.6, 104.6, 81.4, 73.3, 58.4, 56.4, 53.4, 50.6, 47.5, 40.9, 39.9, 35.8, 33.6, 32.9, 29.7, 25.2, 23.5, 3.9. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{32}H_{41}N_8O_2S$: 601.30677; found: 601.30469.

**5.1.11.4. Preparation of (*R*)-8-(3-aminopiperidin-1-yl)-7-(but-2-yn-1-yl)-3-methyl-1-(3-(4-(4-phenyl-1*H*-imidazol-2-yl)piperidin-1-yl)propyl)-1*H*-purine-2,6(3*H*,7*H*)-dione (21d).** Compound **21d** was synthesized from compounds **6d** and **20** according to the general method. Yield: 66.5%. Off-white solid. M.p.: 126-128°C. $[\alpha]_D^{20}$ -16.96 (*c* 1.01, $CHCl_3$). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 7.67 (m, 2H), 7.33 (m, 2H), 7.19 (m, 2H), 4.83 (m, 2H), 4.06 (t, *J* = 7.2 Hz, 2H), 3.60 (m, 1H), 3.52 (m, 1H), 3.50 (s, 3H), 3.03 (m, 4H), 2.83 (m, 2H), 2.48 (m, 2H), 1.65-2.06 (m, 14H), 1.32 (m, 1H). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$: 156.3, 154.6, 152.2, 151.7, 147.8, 142.9, 128.8, 126.8, 124.8, 117.2, 110.1, 104.7, 81.5, 73.3, 58.4, 56.4, 53.5, 50.6, 47.5, 39.9, 36.3, 35.8, 33.6, 31.2, 29.8, 24.8, 23.5, 3.8. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{32}H_{42}N_9O_2$: 584.36560; found: 584.34393.

**5.1.11.5. Preparation of (*R*)-8-(3-aminopiperidin-1-yl)-7-(but-2-yn-1-yl)-3-methyl-1-(3-(4-(5-phenyloxazol-2-yl)piperidin-1-yl)propyl)-1*H*-purine-2,6(3*H*,7*H*)-dione (21e).** Compound **21e** was synthesized from compounds **6e** and **20** according to the general method. Yield: 68.7%. Off-white solid. M.p.: 80-82°C. $[\alpha]_D^{20}$ -18.49 (*c* 1.04, $CHCl_3$). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 7.59 (m, 2H), 7.39 (m, 2H), 7.29 (m, 1H), 7.20 (s, 1H), 4.85 (m, 2H), 4.06 (t, *J* = 6.8 Hz, 2H), 3.61 (m, 1H), 3.54 (m, 1H), 3.50 (s, 3H), 3.04 (m, 4H), 2.86 (m, 2H), 2.52 (m, 2H), 1.69-2.16 (m, 14H), 1.34 (m, 1H). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$: 166.7, 156.2, 154.5, 151.7, 150.9, 147.7, 128.9, 128.4, 128.2, 124.1, 121.7, 104.6, 81.4, 73.3, 58.3, 56.4, 53.0, 50.6, 47.5, 39.8, 35.8, 35.7, 33.5, 29.8, 29.7, 25.3,

23.4, 3.9. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{32}H_{41}N_8O_3$: 585.32961; found: 585.32739.

**5.1.11.6. Preparation of (*R*)-8-(3-aminopiperidin-1-yl)-7-(but-2-yn-1-yl)-3-methyl-1-(3-(4-(5-phenylisoxazol-3-yl)piperidin-1-yl)propyl)-1*H*-purine-2,6(3*H*,7*H*)-dione (21f).** Compound **21f** was synthesized from compounds **6f** and **20** according to the general method. Yield: 68.6%. Yellow solid. M.p.: 122-124°C. $[\alpha]_D^{20}$-15.10 (*c* 1.02, $CHCl_3$). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 7.75 (m, 2H), 7.44 (m, 3H), 6.39 (s, 1H), 4.85 (m, 2H), 4.08 (m, 2H), 3.61 (m, 1H), 3.52 (s, 3H), 3.51 (m, 1H), 3.04 (m, 4H), 2.82 (m, 2H), 2.52 (m, 2H), 2.09 (m, 2H), 1.65-1.96 (m, 14H), 1.30 (m, 1H). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$: 169.7, 168.1, 156.2, 154.5, 151.7, 147.7, 130.1, 129.1, 127.8, 125.9, 104.6, 97.8, 81.4, 73.3, 58.5, 56.5, 53.3, 50.6, 47.5, 39.9, 35.8, 33.5, 31.2, 29.8, 25.1, 23.5, 18.6, 3.8. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{32}H_{41}N_8O_3$: 585.32961; found: 585.32764.

**5.1.11.7. Preparation of (*R*)-8-(3-aminopiperidin-1-yl)-7-(but-2-yn-1-yl)-3-methyl-1-(3-(4-(1-phenyl-1*H*-tetrazol-5-yl)piperidin-1-yl)propyl)-1*H*-purine-2,6(3*H*,7*H*)-dione (21g).** Compound **21g** was synthesized from compounds **6g** and **20** according to the general method. Yield: 36.6%. Yellow solid. M.p.: 117-119°C. $[\alpha]_D^{20}$-13.28 (*c* 1.03, $CHCl_3$).$^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 7.59 (m, 3H), 7.39 (m, 2H), 4.87 (m, 2H), 4.04 (m, 2H), 3.64 (m, 1H), 3.48 (s, 3H), 3.44 (m, 1H), 3.00-3.28 (m, 5H), 2.83 (m, 1H), 2.50 (m, 2H), 2.02 (m, 4H), 1.61-1.85 (m, 12H), 1.30 (m, 1H). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$: 157.9, 155.9, 154.6, 151.7, 147.5, 133.9, 130.8, 130.2, 125.4, 104.7, 81.5, 73.3, 56.4, 56.2, 52.5, 50.8, 47.3, 39.8, 35.7, 31.7, 31.1, 30.1, 29.8, 24.6, 22.7, 3.9. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{30}H_{40}N_{11}O_2$: 586.33610; found: 586.33356.

**5.1.11.8. Preparation of (*R*)-8-(3-aminopiperidin-1-yl)-7-(but-2-yn-1-yl)-3-methyl-1-(3-(4-(3-phenyl-1,2,4-**

**oxadiazol-5-yl)piperazin-1-yl)propyl)-1*H*-purine-2,6(3*H*,7*H*)-dione (21h).** Compound **21h** was synthesized from compounds **16a** and **20** according to the general method. Yield: 76.8%. Off-white solid. M.p.: 181-183°C. $[\alpha]_D^{20}$ -12.60 (*c* 1.08, $CHCl_3$). $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$: 7.99 (m, 2H), 7.43 (m, 3H), 4.88 (m, 2H), 4.08 (t, *J* = 7.2 Hz, 2H), 3.65 (m, 4H), 3.62 (m, 1H), 3.54 (m, 1H), 3.51 (s, 3H), 3.09 (m, 2H), 2.90 (m, 1H), 2.53 (m, 6H), 1.70-1.99 (m, 8H), 1.37 (m, 1H). $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta$: 171.1, 168.8, 156.2, 154.5, 151.7, 147.8, 130.8, 128.7, 128.0, 127.3, 104.6, 81.5, 73.3, 58.0, 56.2, 52.3, 50.7, 47.4, 46.2, 39.8, 35.8, 33.2, 29.8, 25.1, 23.3, 3.9. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{30}H_{39}N_{10}O_3$: 587.32011; found: 587.31818.

**5.1.11.9. Preparation of (*R*)-8-(3-aminopiperidin-1-yl)-7-(but-2-yn-1-yl)-3-methyl-1-(3-(4-(3-phenyl-1,2,4-thiadiazol-5-yl)piperazin-1-yl)propyl)-1*H*-purine-2,6(3*H*,7*H*)-dione (21i).** Compound **21i** was synthesized from compounds **16b** and **20** according to the general method. Yield: 75.5%. Off-white solid. M.p.: 116-118°C. $[\alpha]_D^{20}$ -14.07 (*c* 1.02, $CHCl_3$). $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$: 8.17 (m, 2H), 7.41 (m, 3H), 4.89 (m, 2H), 4.09 (t, *J* = 7.2 Hz, 2H), 3.56 (m, 9H), 3.15 (m, 2H), 2.99 (m, 1H), 2.56 (m, 6H), 1.99 (m, 1H), 1.89 (m, 3H), 1.83 (s, 3H), 1.72 (m, 1H), 1.52 (m, 1H). $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta$: 185.2, 170.5, 156.0, 154.5, 151.7, 147.6, 133.6, 129.9, 128.5, 128.1, 104.6, 81.5, 73.3, 58.5, 56.1, 52.1, 50.7, 49.0, 47.3, 39.7, 35.8, 32.4, 29.8, 25.1, 23.0, 3.9. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{30}H_{39}N_{10}O_2S$: 603.29727; found: 603.29468.

**5.1.11.10. Preparation of (*R*)-8-(3-aminopiperidin-1-yl)-7-(but-2-yn-1-yl)-3-methyl-1-(3-(4-(5-phenyl-4*H*-1,2,4-triazol-3-yl)piperazin-1-yl)propyl)-1*H*-purine-2,6(3*H*,7*H*)-dione (21j).** Compound **21j** was synthesized from compounds **16c** and **20** according to the general method. Yield: 81.3%. Off-white solid. M.p.: 129-131°C. $[\alpha]_D^{20}$ -7.68 (*c* 1.04, $CHCl_3$). $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$: 7.92 (m, 2H), 7.38 (m, 3H), 4.91 (m, 2H), 4.15 (m, 2H), 3.49 (m, 1H), 3.46 (s, 3H), 3.28 (m, 3H), 3.17 (m, 4H), 3.00 (m, 1H), 2.51 (m,

6H), 1.99 (m, 4H), 1.80 (s, 3H), 1.67 (m, 2H). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$: 162.5, 158.4, 155.6, 154.8, 151.8, 147.4, 129.9, 129.6, 128.8, 126.3, 104.9, 81.6, 73.3, 56.4, 55.9, 52.2, 50.9, 47.1, 46.7, 40.2, 35.7, 31.2, 29.8, 24.3, 22.3, 3.8. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{30}H_{40}N_{11}O_2$: 586.33610; found: 586.33362.

**5.1.11.11. Preparation of (*R*)-8-(3-aminopiperidin-1-yl)-7-(but-2-yn-1-yl)-3-methyl-1-(3-(4-(3-methyl-1-phenyl-1*H*-pyrazol-5-yl)piperazin-1-yl)propyl)-1*H*-purine-2,6(3*H*,7*H*)-dione (21k).** Compound **21k** was synthesized from compounds **16d** and **20** according to the general method. Yield: 59.6%. Off-white solid. M.p. 119-121°C, $[\alpha]_D^{20}$ -8.83 (*c* 1.08, $CHCl_3$). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 7.74 (m, 2H), 7.38 (m, 2H), 7.22 (m, 1H), 5.64 (s, 1H), 4.93 (m, 2H), 4.04 (t, *J* = 7.2 Hz, 2H), 3.59 (m, 1H), 3.48 (s, 3H), 3.37 (m, 2H), 3.26 (m, 2H), 2.86 (m, 4H), 2.53 (m, 6H), 2.26 (s, 3H), 1.99 (m, 2H), 1.99 (m, 2H), 1.86 (m, 2H), 1.79 (s, 3H), 1.75 (m, 2H). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$: 155.6, 154.5, 152.4,151.6, 149.0, 147.4, 140.4, 129.0, 126.3, 122.8, 104.8, 94.2, 81.6, 73.3, 56.1, 52.7, 51.0, 47.2, 39.8, 35.7, 29.8, 25.0, 22.5, 14.3, 3.9. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{32}H_{43}N_{10}O_2$: 599.35650; found: 599.35406.

**5.1.11.12. Preparation of (*R*)-8-(3-aminopiperidin-1-yl)-7-(but-2-yn-1-yl)-3-methyl-1-(3-(4-(4-phenylthiazol-2-yl)piperazin-1-yl)propyl)-1*H*-purine-2,6(3*H*,7*H*)-dione (21l).** Compound **21l** was synthesized from compounds **16e** and **20** according to the general method. Yield: 59.8%. Off-white solid. M.p.: 104-106°C. $[\alpha]_D^{20}$ -15.53 (*c* 0.98, $CHCl_3$). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 7.82 (m, 2H), 7.36 (m, 2H), 7.28 (m, 1H), 6.75 (s, 1H), 4.90 (m, 2H), 4.09 (t, *J* = 7.2 Hz, 2H), 3.60 (m, 1H), 3.43-3.51 (m, 8H), 3.19 (m, 2H), 3.01 (m, 1H), 2.55 (m, 6H), 1.83-1.99 (m, 8H), 1.73 (m, 2H), 1.53 (m, 1H). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$: 171.1, 156.1, 154.6, 152.0, 151.7, 147.7, 135.3, 128.6, 127.7, 126.2, 104.6, 101.5, 81.5, 73.3, 57.7, 56.2, 52.4, 50.7, 48.6, 47.4, 39.8, 35.8, 32.9, 29.8, 25.2, 23.2, 3.9. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{31}H_{40}N_9O_2S$: 602.30017; found: 602.30202.

**5.1.11.13. Preparation of (*R*)-8-(3-aminopiperidin-1-yl)-7-(but-2-yn-1-yl)-3-methyl-1-(3-(4-(5-phenyl-1,2,4-oxadiazol-3-yl)piperazin-1-yl)propyl)-1*H*-purine-2,6(3*H*,7*H*)-dione (21m).** Compound **21m** was synthesized from compounds **16f** and **20** according to the general method. Yield: 50.6%. Off-white solid. M.p.: 105-108°C. $[\alpha]_D^{20}$ -9.02 (*c* 0.53, $CHCl_3$). $^1$H NMR (500 MHz, $CDCl_3$) δ 8.06 (d, *J* = 7.5 Hz, 2H), 7.56-7.47 (m, 3H), 5.08-4.88 (m, 2H), 4.09 (t, *J* = 7.0 Hz, 2H), 3.61-3.56 (m, 4H), 3.51 (s, 3H), 3.40 (brs, 2H), 3.31 (brs, 2H), 3.23 (brs, 1H), 2.64 (brs, 6H), 2.04-1.74 (m, 11H). $^{13}$C NMR (125 MHz, DMSO-$d_6$) *δ*: 173.7, 170.1, 155.8, 153.4, 150.8, 147.3, 132.9, 129.4, 127.5, 123.8, 103.3, 81.0, 73.8, 57.2, 55.4, 51.6, 49.7, 47.1, 45.8, 39.8, 35.3, 32.8, 29.3, 24.4, 23.2, 3.1. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{30}H_{39}N_{10}O_3$: 587.32011; found: 587.31708.

**5.1.11.14. Preparation of (*R*)-8-(3-aminopiperidin-1-yl)-7-(but-2-yn-1-yl)-3-methyl-1-(3-(4-(5-methylpyrimidin-2-yl)piperazin-1-yl)propyl)-1*H*-purine-2,6(3*H*,7*H*)-dione (21n).** Compound **21n** was synthesized from compounds **19a** and **20** according to the general method. Yield: 50.1%. Off-white solid. M.p.: 54-56°C. $[\alpha]_D^{20}$ -16.90 (*c* 0.50, $CHCl_3$). $^1$H NMR (400 MHz, $CDCl_3$) δ 8.14-8.13 (m, 2H), 4.92-4.82 (m, 2H), 4.07 (t, *J* = 7.2 Hz, 2H), 3.76-3.73 (m, 4H), 3.65-3.61 (m, 1H), 3.52-3.45 (m, 4H), 3.13-3.06 (m, 2H), 2.95-2.88 (m, 1H), 2.53-2.50 (m, 6H), 2.11 (s, 3H), 2.01-1.90 (m, 4H), 1.82 (t, *J* = 2.0 Hz, 3H), 1.76-1.68 (m, 1H), 1.45-1.37 (m, 1H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ 160.7, 157.7, 155.9, 154.4, 151.6, 147.6, 118.3, 104.5, 81.4, 73.2, 57.3, 56.2, 52.9, 50.6, 47.3, 43.8, 39.6, 35.7, 32.4, 29.7, 25.1, 23.0, 14.6, 3.8. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{27}H_{39}N_{10}O_2$: 535.32520; found: 535.32532.

**5.1.11.15. Preparation of (*R*)-8-(3-aminopiperidin-1-yl)-7-(but-2-yn-1-yl)-1-(3-(4-(5-ethylpyrimidin-2-yl)pi**

**perazin-1-yl)propyl)-3-methyl-1*H*-purine-2,6(3*H*,7*H*)-dione (21o).** Compound **21o** was synthesized from compounds **19b** and **20** according to the general method. Yield: 33.4%. Light-yellow solid. M.p.: 69-70°C. $[\alpha]_D^{20}$ -17.22 (*c* 0.42, $CHCl_3$). $^1H$ NMR (400 MHz, $CDCl_3$) δ 8.15 (s, 2H), 4.90-4.80 (m, 2H), 4.07 (t, *J* = 7.2 Hz, 2H), 3.73 (t, *J* = 4.8 Hz, 4H), 3.64-3.60 (m, 1H), 3.55-3.50 (m, 4H), 3.09-3.03 (m, 2H), 2.90-2.84 (m, 1H), 2.50-2.47 (m, 6H), 2.45-2.41 (m, 2H), 2.00-1.88 (m, 4H), 1.82-1.81 (m, 3H), 1.73-1.69 (m, 1H), 1.40-1.30 (m, 1H), 1.17 (t, *J* = 7.6 Hz, 3H). $^{13}C$ NMR (100 MHz, $CDCl_3$) δ 160.8, 157.1, 156.1, 154.4, 151.6, 147.6, 124.5, 104.5, 81.3, 73.2, 58.0, 56.2, 53.0, 50.5, 47.3, 43.9, 39.7, 35.7, 33.2, 29.6, 25.2, 23.3, 22.7, 15.6, 3.8. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{28}H_{41}N_{10}O_2$: 549.34085; found: 549.33960.

**5.1.11.16. Preparation of (*R*)-8-(3-aminopiperidin-1-yl)-7-(but-2-yn-1-yl)-3-methyl-1-(3-(4-(5-propylpyrimidin-2-yl)piperazin-1-yl)propyl)-1*H*-purine-2,6(3*H*,7*H*)-dione (21p).** Compound **21p** was synthesized from compounds **19c** and **20** according to the general method. Yield: 42.8%. Light-yellow solid. M.p.: 57-60°C. $[\alpha]_D^{20}$ -16.34 (*c* 0.51, $CHCl_3$). $^1H$ NMR (400 MHz, $CDCl_3$) δ 8.13 (s, 2H), 4.91-4.81 (m, 2H), 4.07 (t, *J* = 7.2 Hz, 2H), 3.76-3.75 (m, 4H), 3.65-3.61 (m, 1H), 3.54-3.50 (m, 4H), 3.11-3.06 (m, 2H), 2.92-2.87 (m, 1H), 2.52-2.51 (m, 6H), 2.38 (t, *J* = 7.6 Hz, 2H), 2.01-1.95 (m, 2H), 1.93-1.88 (m, 2H), 1.82 (t, *J* = 2.0 Hz, 3H), 1.77-1.70 (m, 1H), 1.59-1.50 (m, 2H), 1.40-1.37 (m, 1H), 0.92 (t, *J* = 7.6 Hz, 3H). $^{13}C$ NMR (100 MHz, $CDCl_3$) δ 160.8, 157.5, 156.1, 154.4, 151.6, 147.6, 122.9, 104.4, 81.3, 73.2, 58.2, 56.2, 53.0, 50.5, 47.3, 43.9, 39.7, 35.7, 33.3, 31.5, 29.6, 25.2, 24.4, 23.3, 13.5, 3.7. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{29}H_{43}N_{10}O_2$: 563.35650; found: 563.35425.

**5.1.11.17. Preparation of (*R*)-8-(3-aminopiperidin-1-yl)-7-(but-2-yn-1-yl)-1-(3-(4-(5-methoxypyrimidin-2-yl)piperazin-1-yl)propyl)-3-methyl-1*H*-purine-2,6(3*H*,7*H*)-dione (21q).** Compound **21q** was synthesized from compounds **19d** and **20** according to the general method.

Yield: 47.3%. Light-yellow solid. M.p.: 57-60°C. $[\alpha]_D^{20}$ -16.92 (*c* 0.41, $CHCl_3$). $^1H$ NMR (400 MHz, $CDCl_3$) δ 8.08 (s, 2H), 4.91-4.80 (m, 2H), 4.07 (t, *J* = 7.2 Hz, 2H), 3.79 (s, 3H), 3.71-3.68 (m, 4H), 3.65-3.61 (m, 1H), 3.53-3.50 (m, 4H), 3.10-3.05 (m, 2H), 2.92-2.87 (m, 1H), 2.52-2.49 (m, 6H), 2.01-1.95 (m, 2H), 1.93-1.89 (m, 2H), 1.82 (t, *J* = 2.4 Hz, 3H), 1.77-1.68 (m, 1H), 1.42-1.37 (m, 1H). $^{13}C$ NMR (100 MHz, $CDCl_3$) δ 158.0, 156.0, 154.4, 151.6, 147.6, 146.3, 144.9, 104.5, 81.3, 73.2, 57.5, 57.0, 56.2, 52.9, 50.5, 47.3, 44.4, 39.7, 35.6, 32.8, 29.6, 25.2, 23.1, 3.7. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{27}H_{39}N_{10}O_3$: 551.32011; found: 551.31958.

**5.1.11.18. Preparation of (*R*)-8-(3-aminopiperidin-1-yl)-7-(but-2-yn-1-yl)-1-(3-(4-(5-fluoropyrimidin-2-yl)piperazin-1-yl)propyl)-3-methyl-1*H*-purine-2,6(3*H*,7*H*)-dione (21r).** Compound **21r** was synthesized from compounds **19e** and **20** according to the general method. Yield: 46.1%. Off-white solid. M.p.: 72-74°C. $[\alpha]_D^{20}$ -16.50 (*c* 0.53, $CHCl_3$). $^1H$ NMR (500 MHz, $CDCl_3$) δ 8.17 (s, 2H), 4.92-4.83 (m, 2H), 4.07 (t, *J* = 7.2 Hz, 2H), 3.74 (s, 4H), 3.64-3.61 (m, 1H), 3.50 (s, 4H), 3.14-3.10 (m, 2H), 2.97- 2.93 (m, 1H), 2.51 (s, 6H), 2.01-1.98 (m, 1H), 1.92-1.88 (m, 3H), 1.82 (s, 3H), 1.74-1.71 (m, 1H), 1.45-1.43 (m, 1H). $^{13}C$ NMR (100 MHz, $CDCl_3$) δ 158.9, 155.2 (*J* = 167 Hz), 152.8, 151.6, 150.3, 147.6, 145.1 (*J* = 21 Hz), 104.5, 81.3, 73.2, 57.8, 56.2, 52.9, 50.5, 47.3, 44.3, 39.7, 35.7, 33.0, 29.7, 25.2, 23.2, 3.8. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{26}H_{36}FN_{10}O_2$: 539.30013; found: 539.29797.

**5.1.11.19. Preparation of (*R*)-8-(3-aminopiperidin-1-yl)-7-(but-2-yn-1-yl)-3-methyl-1-(3-(4-(5-(trifluoromethyl)pyrimidin-2-yl)piperazin-1-yl)propyl)-1*H*-purine-2,6(3*H*,7*H*)-dione (21s).** Compound **21s** was synthesized from compounds **19f** and **20** according to the general method. Yield: 50.7%. Off-white solid. M.p.: 87-90°C. $[\alpha]_D^{20}$ -16.74 (*c* 0.43, $CHCl_3$). $^1H$ NMR (400 MHz, $CDCl_3$) δ 8.45 (s, 2H), 4.92-4.81 (m, 2H), 4.10-4.06 (t, *J* = 7.2 Hz, 2H), 3.87-3.85 (m, 4H), 3.65-3.61 (m, 1H), 3.56-3.47 (m, 4H), 3.13-3.07 (m, 2H),

2.94-2.89 (m, 1H), 2.51-2.48 (m, 6H), 2.01-1.83 (m, 4H), 1.83 (m, 3H), 1.77-1.68 (m, 1H), 1.44-1.39 (m, 1H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ 162.1, 156.0, 155.4, 154.3, 151.6, 147.6, 124.3 (q, *J* = 268 Hz), 112.7 (q, *J* = 34 Hz), 104.5, 81.4, 73.2, 57.6, 56.1, 52.8, 50.6, 47.3, 43.9, 39.7, 35.7, 32.8, 29.7, 25.2, 23.1, 3.8. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{27}H_{36}F_3N_{10}O_2$: 589.29693; found: 589.29626.

**5.1.12. Preparation of (*R*)-8-(3-aminopiperidin-1-yl)-7-(but-2-yn-1-yl)-3-methyl-1-(3-(4-(3-phenyl-1,2,4-oxadiazol-5-yl)piperazin-1-yl)propyl)-1*H*-purine-2,6(3*H*,7*H*)-dione hydrochloride (22)**

The gram scale-up synthesis of **21h** was according to the procedure 5.1.11.8. To a solution of **21h** (1.2 g, 2.05 mmol) in EtOH (80 mL) was added 1mol/L HCl/EtOH solution (1.84 mL, 1.84 mmol) dropwise. The mixture was stirred at room temperature for 5 h, then filtered. The filtrate was evaporated *in vacuo* to give a white solid. The white solid was washed with 30 mL $Et_2O$ under refluxing condition for 1h. After cooling to the ambient temperature, the white solid was separated by filtration, washed with $Et_2O$ (2×5 mL) and dried to afford **22** (1.0 g, 87.2% yield). White solid. M.p.: 144-146°C. $^1$H NMR (400 MHz, $CDCl_3$) *δ*: 8.67 (brs, 2H), 7.98-7.96 (m, 2H), 7.45-7.41 (m, 3H), 5.09-4.92 (m, 2H), 4.07 (t, *J* = 7.2 Hz, 2H), 3.74-3.69 (m, 5H), 3.62-3.59 (m, 1H), 3.53-3.45 (m, 5H), 3.29 (brs, 1H), 2.69 (brs, 6H), 2.06 (brs, 3H), 1.96-1.93 (m, 2H), 1.84 (s, 3H), 1.76 (brs, 1H). $^{13}$C NMR (100 MHz, $CDCl_3$) *δ*: 171.0, 168.7, 154.8, 154.5, 151.5, 147.0, 130.8, 128.7, 127.9, 127.3, 104.9, 81.9, 73.2, 56.1, 52.5, 52.2, 51.5, 46.9, 46.0, 39.8, 35.6, 29.9, 27.8, 25.0, 21.4, 4.0. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{30}H_{39}N_{10}O_3$: 587.32011; found: 587.31854.

**5.1.13. X-ray crystallographic structure of compound 21h**

Single crystals of compound **21h** were obtained from a mix solution of methanol and dichloromethane (V/V = 4:1) and characterized by X-ray diffraction. The single-crystal X-ray structure of compound **21h** is depicted in **Fig. 3**. The lattice

parameters were a =11.343(3), b =14.695(3), c = 18.029(4)Å, β = 96.296(5)°, and the lattice volume was 2987.1(12)Å$^3$. There were four molecules in one lattice.

### 5.2. Biological evaluation

#### 5.2.1. DPP-IV inhibition assay

DPP-IV inhibition assay was performed as described previously [27]. Briefly, 10 μL of compound solution *(the final concentrations of compound were presented in indicated figures and legends)* was added into 100 μL of enzyme reaction system containing 2 mU/mL DPP-IV enzyme and 0.26 mmol/L Gly-Pro-p-nitroanilide, the changed absorbance (△OD) at $OD_{405nm}$ was detected before and after one hour of incubation at 37℃, then the percentage of inhibition was calculated as: Inhibition (%) = $(\triangle OD_{control} - \triangle OD_{compound})/\triangle OD_{control} \times 100\%$. The $IC_{50}$ value represents the concentration of each compound resulting in 50% maximum inhibition of DPP-IV.

#### 5.2.2. DPP-8/9 inhibition assay

DPP-8/9 inhibition assay was performed by using recombinant human DPP-8/9 protein and a DPP-8/9 over expressing cell model established in our laboratory as described previously [27, 28]. The enzyme reaction was similar as DPP-IV reaction mentioned above, only the concentrations of enzyme and substrate were different.

#### 5.2.3. GPR119 agonism assay

Plasmids construction of human GPR119, construction fused ligand binding domain of CREB (CREB-LBD) with DNA binding domain of Gal4 (Gal4-DBD), and construction of Gal4 upstream activator sequence (UAS)-triggered luciferase reporter were all established in our laboratory. To detect potential transactivation of the indicated compound to GPR119, HEK293 cells were transiently transfected using Lipofectamine 2000 (Life Technologies) with plasmids constructions mentioned above, following treated with compounds at designated concentrations and DMSO as vehicle, then chemiluminescence value reflecting to luciferase activity was measured

by a microplate reader (Biotek, synergy 2) using the firefly-luciferase assay kit (Vigorous Biotechnology, Beijing). The activation fold was calculated as value $_{compound}$ / Value $_{vehicle}$, and relative activity percentage of indicated compounds was normalized to the activation fold of positive control, AR231453. The $EC_{50}$ of GPR119 agonism was obtained by GraphPad Prism software.

**5.2.4. Pharmacokinetic studies**

All animal protocols were approved by Institute Animal Care and Use Committee. The selected compound **21h** was subjected to pharmacokinetic studies in ICR mouse (male) weighing 26 to 27 g with three mice in oral administration group and three mice in intravenous injection group. The tested compound was formulated at a concentration of 2 mg/mL for a dose of 20 mg/kg given orally (p.o.) and at 0.4 mg/mL for a dose of 2 mg/kg given intravenously (i.v.). The tested compound was formulated with 0.5% carboxymethyl cellulose for p.o. administration and with 30% SBECD with 1 mol/L HCl for i.v. administration. Blood samples were collected at 5, 15, 30 min, 1, 2, 4, 7, 24 h after oral dosing and i.v. administration. Plasma was harvested and stored at -80 °C until analysed.

Compounds **21h** and **22** were subjected to pharmacokinetic studies in SD rat (male) weighing 180 to 200 g with three rats in oral administration group and three rats in intravenous injection group. The tested compounds were formulated at a concentration of 3.186 mg/mL (**22**) and 3 mg/mL (**21h**) for a dose of 30 mg/kg (free base content) given orally (p.o.) and at 0.638 mg/mL (**22**) for a dose of 3 mg/kg (free base content) given intravenously (i.v.). The tested compounds were formulated with 0.5% carboxymethyl cellulose for p.o. administration and with normal saline for i.v. administration. Blood samples were collected at 5, 15, 30 min, 1, 1.5, 2, 4, 6, 8, 12, 24 h after oral dosing and i.v. administration. Plasma was harvested and stored at -80 °C until analysed.

The pharmacokinetic parameters were calculated using WinNonlin software version 6.3 based on non-compartmental analysis (Pharsight Corporation, Mountain

View, USA). The oral bioavailability was calculated as the ratio between the area under the curve (AUC) following intravenous administration corrected for dose ($F = (AUC_{p.o.} \times dose_{i.v.})/(AUC_{i.v.} \times dose_{p.o.})$).

### 5.2.5. Oral glucose tolerance test (OGTT)

To analyze the acute effect of **21h** and **22** on glucose excursion *in vivo*, the OGTT was performed in ICR mice as following: In one experiment, the overnight-fasted mice were administered by oral gavage with distilled water (vehicle), **21h** (30 mg/kg), **22** (equal molar dose compared to **21h,** 32 mg/kg). In another experiment, the overnight-fasted mice were administered by oral gavage with distilled water (vehicle), **22** (30 mg/kg), sitagliptin (10 mg/kg), APD597 (10 mg/kg) and linagliptin (2 mg/kg) prior to the oral glucose load (2 g/kg). Blood samples were collected from the tail tip before the glucose load (0 min) and at 30, 60, 120 min after the glucose load to measure blood glucose levels using the glucose oxidase method.

### 5.2.6. Caco-2 permeability assay

The Caco-2 cell line is derived from a human colon adenocarcinoma and has been widely used as an in vitro model for the prediction of intestinal drug permeability and absorption [29]. The cells were seeded in Transwells (Millipore, 0.4 μm pore size) and formed a confluent monolayer after 21 days culture. On day 21, the target compound 21 (50 μM) was added into the apical side of the membrane and the concentration of the compound across Caco-2 cell monolayer was quantified by UPLC after 1 hour incubation at 37℃. Transepithelial electrical resistance (TEER) should be determined before as well as after transport experiments and should be over $500\Omega \times cm^2$. The apparent permeability coefficient (Papp) for the compound is calculated from the following equation: ($P_{app} = (dQ/dt)/(C_0 \times A)$).

Where dQ/dt is the rate of permeation of the drug across the cells, $C_0$ is the initial concentration and A is the area of the cell monolayer.

**5.2.7. Mouse liver microsome stability assay**

Mouse liver microsome stability assay was performed as described previously [30]. Briefly, mouse liver microsomal incubation contained microsomal protein (0.5 mg/mL), target compound **22** (10 μmol/L), Tris-HCl buffer and a NADPH-generating system. Incubations in duplicate were performed at 37 ℃ for 30 min. Reactions were initialed by addition of NADPH-generating system and terminated by adding equal volume of ice-cold acetonitrile which contained internal standard. The mixtures were centrifuged and the supernatants were analyzed by LC-MS/MS. Incubations with inactive liver microsomes served as negative controls.

**5.2.8. Hepatocyte stability assay.**

The assay was performed with hepatocytes from male ICR/CD-1 Mouse (BioreclamationIVT), Male Sprague-Dawley Rat (BioIVT) and mixed human (BioIVT) following the protocol as described previously [30]. The assay evaluated the metabolic stability of compounds in hepatocytes by measuring amount of parent remaining of the test compounds.

**5.2.9. Plasma protein binding**

The extent of plasma protein binding for the tested compound was determined by equilibrium dialysis. Compound **22** was added to pre-warmed (37 °C) human plasma and mixed (5 μM, 1% (vol/vol) DMSO). Dialysis plate was prepared by adding 350 μL of phosphate buffer (1% (vol/vol) DMSO) to the buffer compartment and 200 μL of the tested compound plasma solution (5 μM) to the red compartment. After incubating the plate at 37 °C for 5 hours at 100 rpm on orbital shaker, samples were removed from each compartment for LC-MS/MS analysis.

**5.2.10. Cytotoxicity assay**

Vero cells was cultured in RPMI1640 medium supplemented with 10% fetal bovine serum (FBS). The cells were incubated in a humidified atmosphere of 5% $CO_2$

at 37 °C. Stocks of cells were cultured in 25-$cm^2$ tissue culture flasks and subcultured two to three times per week. Cytotoxicity testing was performed in a transparent 96-well microplate. Outer perimeter wells were filled with sterile water to prevent dehydration in experimental wells. The cells were incubated at 37 °C under 5% $CO_2$ until confluent and then diluted with culture medium to $4 \times 10^5$ cells/mL. Threefold serial dilutions of the stock solutions resulted in final concentrations of 64 to 0.26 μg/mL in a final volume of 100 μL. After incubation at 37 °C for 48 h, the medium was removed, and the monolayers were washed twice with 100 μL of warm Hanks balanced salt solution (HBSS). Warm medium (100 μL) and 10 μL of freshly made methyl-thiazolyldiphenyl-tetrazolium bromide (MTT) were added to each well, and then the plates were incubated for 4 h, after which the absorbance was determined at 492 nm.

**5.2.11. Acute toxicity study**

Compound **22** was screened *in vivo* with a single dose in ICR mouse (female) weighing 18 to 21 g with ten mice. The number of mice which survived after an oral administration of a single dose at 1.5 g/kg, followed by a 7-day observation, was recorded.

**5.2.12. Mini-Ames test**

The mutagenic activities of compound **22** were detected accroding to the published protocol [31, 32]. Two strains of Salmonella typhimurium bacteria (TA98, TA100) were used in the study and were originally obtained from Molecular Toxicology (Boone, NC), USA. DMSO was used as the solvent/vehicle control and the highest concentration tested was 1000 μg/well, which was equal to the OECD limit concentration of 5000 μg/plate in standard Ames assay. Bacteria, test compound or vehicle/positive control formulation, and 10% S9 mixture or PBS buffer were added to molten agar at 45 °C, mixed rapidly, and poured onto 6-well plate containing minimal agar media. After the agar was solidified, the plates were inverted and

incubated at 37 °C for 48-72 hours. Revertant colonies were counted manually and the background lawn was inspected for signs of cytotoxicity. The results were considered to be positive for mutagenic potential if the increase in mean revertants at the peak of the dose response was equal to or greater than 2-fold the mean solvent/vehicle control value and the increase should be dose related. The results indicated that compound **22** was not cytotoxic to the tester strains and was not mutagenic at concentrations up to 1000 μg/well.

### 5.2.13. Inhibition evaluation on hERG $K^+$ channel

The electrophysiology recording of hERG channel current was carried out following the protocol as described previously [33]. A stable cell line of wild-type Chinese hamster ovary cells expressing hERG potassium channels (CHO/hERG) was maintained in culture medium. Cells were cultured for at least 24 hours and then were used for electrophysiological recordings. Voltage-clamp recording was performed in the whole cell patch-clamp configuration. Whole-cell recordings were analyzed using Pulse Fit (V8.74, Heka Electronic, Lambrecht, Pfalz, Germany). Figures were plotted using Origin (7.5 Origin Lab Co., MA, USA). All experiments were performed in duplicate for $IC_{50}$ determination.



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- Novel xanthine derivatives were designed through ring formation strategy.
- Systematic optimization from previous hit 20i led to the new lead compound HBK001.
- HBK001 had potent DPP-IV inhibition and moderate GPR119 agonism activity.
- HBK001 hydrochloride with superior PK showed potent *in vivo* glucose-lowering effect.

**Declaration of interests**

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: