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Original article

Synthesis and structure–activity relationship of non-phosphorus-based fructose-1,6-bisphosphatase inhibitors: 2,5-Diphenyl-1,3,4-oxadiazoles

Ben-Ren Liao [a, 1], Hai-Bing He [a, c, 1], Ling-Ling Yang [b, 1], Li-Xin Gao [b], Liang Chang [a], Jie Tang [a], Jing-Ya Li [b, *], Jia Li [b, *], Fan Yang [a, *]

[a] Shanghai Engineering Research Center of Molecular Therapeutics and New Drug Development, Department of Chemistry, East China Normal University, Shanghai 200062, China
[b] National Center for Drug Screening, Shanghai Institute of Materia Medica, Chinese Academy of Science, Shanghai 201203, China
[c] Chemistry and Chemical Engineering, Nantong University, Jiangsu 226019, China

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ABSTRACT

With the aim of discovering a novel class of non-phosphorus-based fructose-1,6-bisphosphatase (FBPase) inhibitors, a series of 2,5-diphenyl-1,3,4-oxadiazoles were synthesized based on the hit compound (**1**) resulting from a high-throughput screening (HTS). Structure–activity relationship (SAR) studies led to the identification of several compounds with comparable inhibitory activities to AMP, the natural allosteric inhibitor of FBPase. Notably, compound **22** and **27b**, bearing a terminal carboxyl or 1*H*-tetrazole, demonstrated remarkable inhibition to gluconeogenesis (GNG). In addition, both inhibition and binding mode to the enzyme were investigated by enzymatic kinetics and in silico experiments for representative compounds **16** and **22**.

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1. Introduction

Gluconeogenesis (GNG), a predominant factor of increased hepatic glucose output [1–4], is a highly regulated process catalyzed by several gluconeogenic enzymes such as fructose-1,6-bisphosphatase (FBPase) which is one of the rate-limiting enzymes [5]. Comparing to the other two rate-limiting enzymes (PEPCK and G6Pase) in multiple roles in GNG, FBPase functions only within the GNG pathway by catalyzing the conversion of D-fructose-1,6-bisphosphate (FBP) to D-fructose-6-phosphate (F6P) [6]. Inhibition of FBPase would be expected as a reasonable way by suppressing GNG to decrease plasma glucose. Actually, in insulin-deficient and insulin-resistant animal models of diabetes, liver FBPase activity is elevated, highlighting the importance of this enzyme in the control of blood glucose [7]. Additionally, in view of its position in the GNG pathway, FBPase shows more attraction as a drug target, which enables inhibition of GNG from all GNG substrates while avoiding direct effects on glycogenolysis, glycolysis, and the tricarboxylic acid cycle as well [8,9].

It has been clarified that the regulation of FBPase activity involves changes in quaternary structure between the active (R) and inactive (T) conformational states [10,11]. FBPase is naturally inhibited by AMP by acting on the allosteric binding site (composed of a hydrophilic phosphate binding site and a hydrophobic pocket), inducing the enzymatic shifting from R to T conformation. Moreover, substrate analogue fructose-2,6-bisphosphate, a potent competitive inhibitor of FBPase, synergistically increases the binding affinity of AMP [12–14]. For decades, significant effort has been brought to develop small-molecular inhibitors against FBPase, focusing on either substrate binding site [15] or AMP binding site [16–25]. Although several series of compounds with FBPase inhibitory activity were reported, such as anilinoquinazolines [16,17], indole carboxylic acids [18], benzenesulfonamides [19,20], sulfonylureas [22,23], tricyclic compounds [24] and achyrofurans [25], few of them achieved such a success as CS-917, the prodrug of MB05032 (Chart 1), which inhibits the enzyme by interacting with the AMP binding site through the phosphate group [6,21,26–28].

* Corresponding authors.
E-mail addresses: jyli@mail.shcnc.ac.cn (J.-Y. Li), jli@mail.shcnc.ac.cn (J. Li), fyang@chem.ecnu.edu.cn (F. Yang).
[1] These authors contributed equally to this work.

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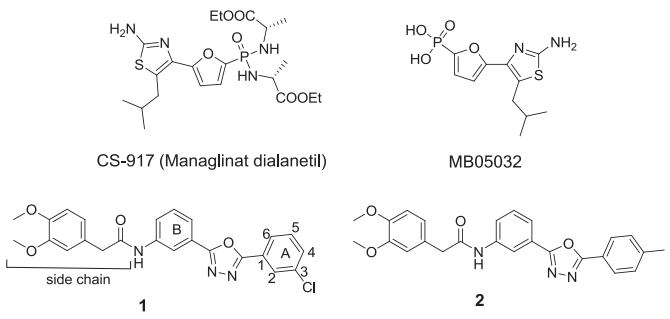


Chart 1. The previously reported inhibitors of FBPase.

Nevertheless, besides FBPase, AMP also modulates a number of other enzymes controlling different biological functions [29]. Thus it is of great attraction to develop new potent FBPase inhibitors which are structurally distinct from AMP and likely bind to the enzyme at least partly by a different mode.

With the intention of searching for new hits with FBPase inhibitory activity, we carried out a high-throughput screening (HTS) campaign of a lead-like library of small molecular compounds, resulting in the identification of **1** endowed with a good drug likeness according to Lipinski's rule [30,31]. Compound **1**, inhibiting FBPase with the IC_{50} value of 15.45 μM at molecular level, was characterized as 2,5-diphenyl-1,3,4-oxadiazoles structure type. In previous report the preliminary SAR of derivatives of **1** by modifying aromatic group A was disclosed, resulted in the discovery of a three-fold more potent compound **2** (4.51 ± 0.22 μM) [32]. Herein, we report further evolution of the novel non-phosphorus FBPase inhibitors, mainly focused on the modification on the side chain of compound **2** (Chart 1). Their effects on glucose output in rat hepatocytes were explored for the compounds that showed stronger potency at molecular level. Furthermore, both the inhibition and binding modes to the enzyme are discussed.

2. Chemistry

All the target compounds were obtained via an amidation reaction of *m*eta-amino substituted diphenyl-1,3,4-oxadiazole **8** with kinds of acids, acyl chlorides or acetic anhydride. Compound **8** was prepared via a classical procedure: dehydrated cyclization of dihydrazide **6** with $POCl_3$ in acetonitrile followed by the Raney-Ni-catalyzed hydrogenation of the nitro to amino group on aromatic ring B (Scheme 1) [33].

The reaction of **8** with acetic anhydride afforded **9** conveniently, while **10**–**13** were prepared from the reactions of **8** with appropriate acyl chlorides (Scheme 2).

The synthesis of the compounds bearing hydroxyl groups at the end of the side chain is illustrated in Scheme 3. Different pathways were employed in the light of various lengths of the alkyl chains. The reaction of **8** with 6-hydroxyhexanoic acid (**16a**) using EDC as the condensation agent gave **16** directly. In contrast, due to the instabilities of 4-hydroxybutanoic acid and 5-hydroxypentanoic acid, compounds **14** and **15** were obtained from **8** via a relatively complicated three-step procedure, including acylation with anhydride, methylation with iodomethane and reduction of the terminal ester group to hydroxyl group (step d, e and c). **17**, **18**, **19** and **20** were also prepared by the reduction of the corresponding terminal ester group with $NaBH_4$ in variable yields [34]. However, different from **14a** and **15a**, the related ester intermediates **17a**–**20a** were synthesized by the reactions of **8** with the corresponding diacid monoesters in one step. The vicinal diol group of compound **21** was formed by oxidation of the terminal alkenyl group of **21a** with $K_3Fe(CN)_6$ and $K_2OsO_2(OH)_4$ [35] and **21a** was generated conveniently from the condensation of **8** with hex-5-enoic acid. Compound **22**, which bears a terminal carboxyl group, was synthesized by the reaction of **8** with pimelic acid in the presence of EDC and HOBt in DCM.

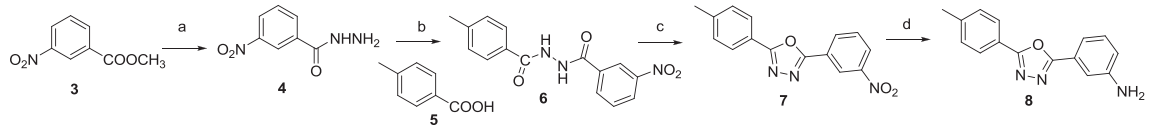
The synthesis of the compounds containing at least one tertiary amine unit as the hydrogen bond acceptor is displayed in Scheme 4. The introduction of amine unit was carried out by replacement of the terminal halogen with a secondary amine, including morpholines (**25a**, **25c** and **25i**), piperidine (**25b**), 4-hydroxypiperidine (**25f**), piperazine (**25e**) and N-methyl piperazines (**25d** and **25h**). However, due to the poor leaving property of chlorine, it was necessary to previously replace the chlorine on **23** with iodine by using NaI (step b) [36]. Acetylation of **25e** with acetic anhydride readily yielded **25g**, whereas the ethyloic derivatization on the piperazine ring of **25e** was accomplished via a winding way which was starting from **24** by coupling with *tert*-butyl-2-(piperazin-1-yl) acetate **28** (step h) [37] and followed by deprotection in TFA/DCM (step i) [38]. As the classical isostere of carboxyl, 5-substitued tetrazole (**27b**) was synthesized from a cyano group (**27a**) with Me_3SiN_3 at 120 °C using NMP as the solvent under microwave assistance. The intermediate **27a** was obtained conveniently by reacting **24** with Me_3SiCN [39].

3. Results and discussion

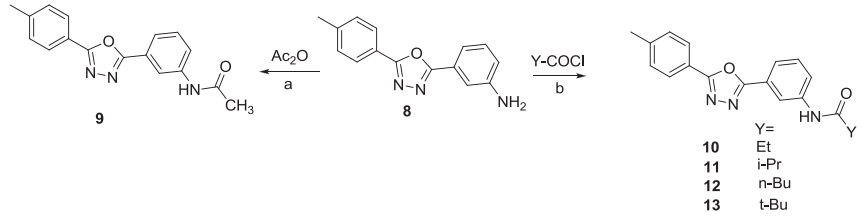
3.1. Activities at molecular level

In vitro FBPase (recombinant human FBPase) inhibition assays were carried out using a coupled spectrophotometric method reported by Doris Rittmann [40], and AMP, the natural allosteric inhibitor to FBPase, served as a positive control in the experiments (IC_{50} = 1.3 ± 0.44 μM). On the basis of our previous work, 2,5-diphenyl-1,3,4-oxadiazole scaffold with 4-methyl group on aromatic ring A was assayed as the optimum structure, thus the side chain of the molecule would be the second key moiety to be explored. At first, we attempted to replace the side chain (including various terminal groups) with simple alkyl group without any change of the amido bond which is usually considered to play a key role in binding to enzymes via hydrogen bond interactions. When the 3,4-dimethoxybenzyl unit was replaced with methyl group, compound **9** kept a comparable inhibition potency (IC_{50} = 7.70 ± 0.34 μM) to **2**. However, along with the lengthening or enlarging of the alkyl chain (Table 1, **10**–**13**), inhibition potencies of the corresponding compounds decrease. We speculated that a group with strong hydrophobic property (a long or bulky alkyl) would be disadvantageous to the binding with FBPase. On the other hand, it also seemed disobedient that **2**, bearing the largest side chain, displayed the best activity comparing to the other compounds in Table 1. It is suggested that the two methoxy groups on the side aromatic ring in **2** contributed a lot to the interactions with FBPase, probably acting as a hydrogen bonding acceptor, and thus it can be inferred that a side chain composed of a small alkyl chain having a hydrogen bonding acceptor would be beneficial to the activity upon the enzyme.

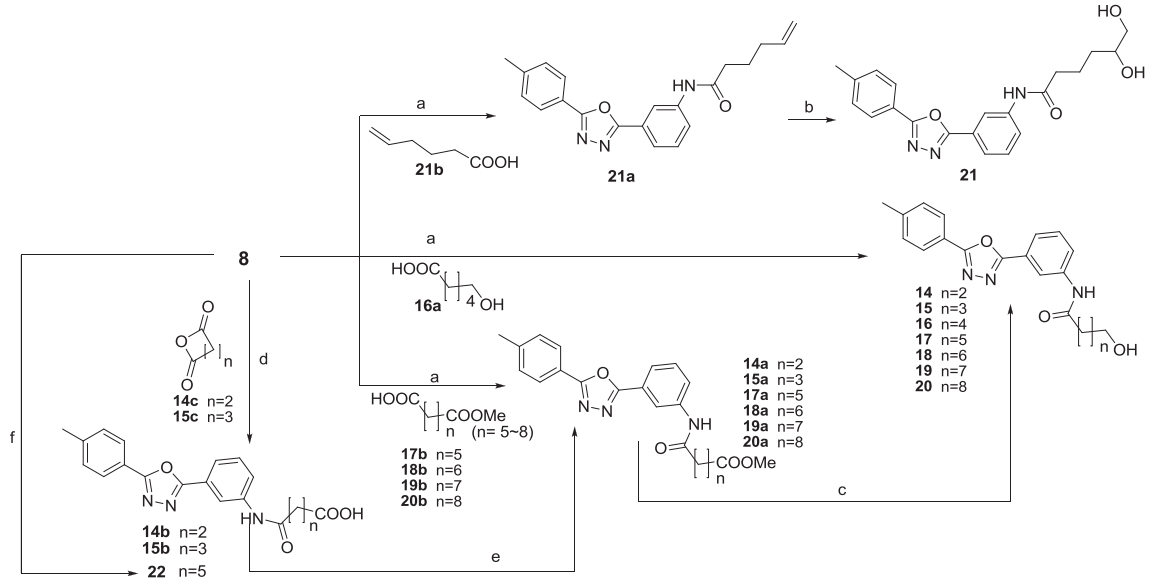
Since a small alkyl chain with a hydrogen bonding acceptor was considered to improve the inhibition against FBPase, linear alkyls with terminal hydroxyl group (Table 2) or amino (Table 3) were designed and synthesized for exploring. As outlined in Table 2, most of the compounds with a hydroxyl group at the end of the alkyl chain inhibited the enzyme significantly, and the potencies varied with respect to the lengths of the alkyl chains. With the increase of carbons from 4 (**14**) to 6 (**16**), the corresponding inhibition potencies increased synchronously till about 12-fold more



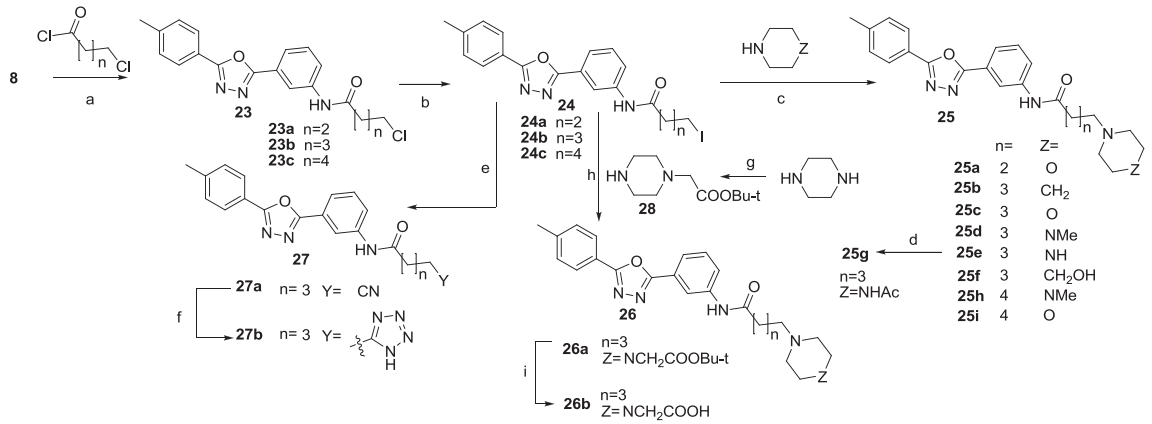
Scheme 1. Reagents and conditions: (a) Dioxane, 85% hydrazine hydrate, reflux, 6 h, (99%); (b) EDC, HOBt, 3-picoline, DCM, RT, 12 h, (65–75%); (c) $POCl_3$, CH_3CN, reflux, 8 h; (d) Raney-Ni, H_2, 1 atm, RT, overnight.



Scheme 2. Reagents and conditions: (a) 3-picoline, DCM, 0–8 °C, 8 h; (b) 3-picoline, DCM, −5 °C ~ 5 °C, 3 h.

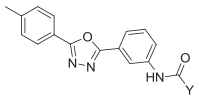


Scheme 3. Reagents and conditions: (a) EDC, HOBt, DCM, 3-picoline, RT, 16–30 h; (b) $K_3Fe(CN)_6$, K_2CO_3, $K_2OsO_2(OH)_4$; (c) $NaBH_4$, THF, MeOH, reflux; (d) Et_3N, THF, RT; (e) MeI, K_2CO_3, acetone, RT, 20 h; (f) Pimelic acid, EDC, HOBt, DCM, 3-picoline, RT.



Scheme 4. Reagents and conditions:(a) Appropriate chloroalkanoyl chloride, 3-picoline, DCM, 0 °C ~ RT, overnight; (b) NaI, dried acetone, reflux, 12 h; (c) THF, RT, overnight; (d) Ac_2O, Et_3N, 0 °C ~ RT, 4 h; (e)Me_3SiCN, Bu_4NF, CH_3CN, RT, 24 h; (f) Me_3SiN_3, NMP, 120 °C, microwave, 30 min; (g) $BrCH_2COOBu$-t, EtOH, 20 h; (h) K_2CO_3, THF, CH_3CN, RT, 36 h; (i) CF_3COOH, DCM, 0 °C ~ RT, 4 h.

Table 1
IC_{50} values of compounds **11** ~ **13** against FBPase.



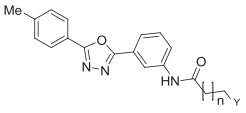
Compd.	Y	FBPase, IC_{50} (μM)[a]
2		4.51 ± 0.22
9	Me	7.70 ± 0.34
10	Et	27.15 ± 0.63
11	*i*-Pr	>50
12	*n*-Bu	33.80 ± 0.55
13	*t*-Bu	>50

[a] IC_{50} values were determined by regression analyses and expressed as means ± SE of three replicates.

potent (**16**, IC_{50} = 1.32 ± 0.21 μM) than that of the hit compound **1**. However, further lengthening the chain reduced the inhibitory activity which was almost lost when the number of carbons added up to 9 (**19**) and 10 (**20**). The intermediates **14a**, **14b** and **15**, bearing a hydrogen bonding acceptor of carboxyl or methyl carboxylate group, also exhibited moderate inhibitions against FBPase. Despite the poor potency of **14b** (IC_{50} = 42.90 ± 3.22 μM), its better water solubility inspired us to synthesize compound **22** with an optimized length of side chain. Encouragingly, **22** demonstrated an 8-fold more potent activity (IC_{50} = 5.32 ± 0.59 μM) than **14b**. Therefore, it was not surprising that the double hydroxyls substituted compound **21** showed a better activity.

Azacyclohexanes, including piperidine, piperazine and morpholine which usually furnish a structure with amphipathicity (hydrophilicity & hydrophobicity) and potential hydrogen bonding interactions, are widely incorporated into drug molecules. IC_{50} values of the compounds with a terminal azacyclohexane in replace of hydroxyl are exhibited in Table 3. In contrast to piperidine (**25b**, IC_{50} = 14.20 ± 2.01 μM), morpholines (**25a**, **25c** and **25i**), piperazine (**25e**) or N-methyl piperazines (**25d** and **25h**) which are composed of two heteroatoms, inhibited FBPase more potently. Furthermore, length of the alkyl chain also affected the activities obviously. For example, **25i** showed an improved two-fold activity than **25c**. Comparing with **25c** (IC_{50} = 5.01 ± 0.55 μM), replacement of morpholine with 4-hydroxypiperidine (**25f**) led to approximate three-fold decrease of the activity. Acetylation of the terminal piperazine **25e** (IC_{50} = 3.97 ± 0.50 μM) yielded **25g** which exhibited a similar activity. On account of the enhanced water solubility brought by carboxyl, an ethyloic group was incorporated to **25e**, affording **26b** which displayed a half inhibitory activity. Another strategy for applying the property of carboxyl is to introduce its isosteres of which tetrazole (**27b**) is the most popular owing to a similar pKa value and multiple hydrogen bond acceptors that would be beneficial to the binding with FBPase. Fortunately, the obtained compound **27b** with a terminal tetrazole demonstrated an inhibitory activity (IC_{50} = 2.98 ± 0.35 μM) which is as good as those of terminal hydroxyl or azacyclohexyl compounds.

Table 2
IC_{50} values of compounds **14** ~ **22** against FBPase.



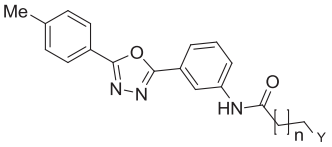
Compd.	*n*	Y	FBPase, IC_{50} (μM)[a]
14	2	OH	5.61 ± 0.56
15	3	OH	3.59 ± 0.42
16	**4**	**OH**	**1.32 ± 0.21**
17	5	OH	3.06 ± 0.37
18	6	OH	11.25 ± 1.13
19	7	OH	>50
20	8	OH	>50
14a	1	−COOMe	7.99 ± 1.01
14b	1	−COOH	42.90 ± 3.22
15a	2	−COOMe	10.68 ± 1.55
21	2	$-CHOHCH_2OH$	3.51 ± 0.39
22	4	−COOH	5.32 ± 0.59

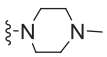
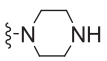
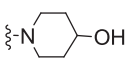
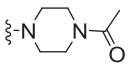
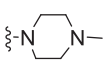
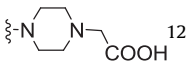
[a] IC_{50} values were determined by regression analyses and expressed as means ± SE of three replicates.

3.2. Inhibition of glucose output in rat hepatocytes

In order to further determine the inhibitory ability to FBPase of the compounds which showed good potencies at molecular level,

Table 3
IC_{50} values of compounds **25** ~ **27** against FBPase.



Compd.	*n*	Y	FBPase, IC_{50} (μM)[a]
25a	2		4.96 ± 0.48
25b	3		14.20 ± 2.01
25c	3		5.01 ± 0.55
25d	3		3.92 ± 0.41
25e	3		3.97 ± 0.50
25f	3		13.20 ± 2.11
25g	3		3.58 ± 0.42
25h	4		2.30 ± 0.30
25i	4		2.54 ± 0.39
26b	3		7.12 ± 0.85
27b	3		**2.98 ± 0.35**

[a] IC_{50} values were determined by regression analyses and expressed as means ± SE of three replicates.

Table 4
Inhibition of glucose output in rat hepatocytes under FBPase inhibitors.[a,b]

Compd.	10 μM (%)	20 μM (%)	40 μM (%)
Metformin[c]	75–87[d]		
2	96.7	93.5	103.2
9	96.8	98.6	86.1
16	89.0	95.3	96.8
21	88.7	91.9	96.7
22	81.9	91.0	75.6
25e	100	98.4	96.8
25g	90.3	91.9	96.7
25h	95	93.6	56.5
25i	83.3	83.6	85.2
27b	89.8	85.9	74.2

[a] The ratio of the produced glucose concentration under inhibitors into the blank control (0.1% DMSO).
[b] Means of three parallel wells.
[c] control, at 2 mM.
[d] The ratio of the produced glucose concentration into the blank (0.1% DMSO).

their effects on the glucose production in primary rat hepatocytes were explored with the existence of sodium pyruvate (2 mmol/L) as the GNG substrate [41]. Metformin was used as the control, because it is recognized as the only marketed drug that acts, at least partially, through inhibition of GNG [42]. As illustrated in Table 4, five compounds (**9**, **22**, **25h**, **25i** and **27b**) inhibited the glucose production in hepatocytes effectively at 40 μM. Actually, for compound **22** and **25i**, remarkable inhibition was obtained even at 10 μM (81.9% and 83.3% respectively). Of all the compounds, **25h** showed the most potent inhibition of glucose output, up to 56.5% at 40 μM. However, comparing to the blank control group (0.1% DMSO), total proteins in the three parallel wells containing **25h** at 40 μM were significantly lower (68.7%, 65.0% and 73.4% of blank group independently), which may be because the reduction of glucose production by **25h** is mainly associated with its cytotoxicity to rat hepatocytes. Additionally, the decrease of total proteins was not observed for the other compounds brought into the assays. Therefore, for the sake of investigating detailed inhibition potencies of the four compounds (**9**, **22**, **25i** and **27b**), we carried out further assays at six concentrations (5–160 μM) [Fig. 1]. In spite of an approximate 15% inhibition at 40 μM, increasing the concentration of **9** failed in improving the potency, which was well explained by the precipitate of **9** at 80 μM and 160 μM. Similar phenomenon appeared when testing **25i**, which might take the responsibility for the dramatic rise of glucose concentration at 80 μM and 160 μM. Fortunately, the inhibition effect against glucose output by compound **22** and **27b** was found to be concentration-dependent, especially for **27b**. At 160 μM, **22** and **27b** suppressed the glucose production about 42% and 59% independently, and the corresponding calculated IC_{50} values were 167.3 (±0.11) μM and 112.5 (±0.04) μM respectively. Both of the two compounds demonstrated good water solubility as well as excellent membrane permeability, which enable the compounds to be promising candidates for development of drugs against T2DM.

3.3. Characterization of the inhibitors on enzyme kinetics

For evaluating the inhibitory modality of the compounds, compound **16** was selected for enzymatic kinetic study on FBPase. As shown in Fig. 2(a), activity of the enzyme recovered gradually after diluting the incubation mixture of FBPase and **16**, and the activity recovered about 90 percent just over 10 h. The result suggests that inhibition of FBPase by **16** is a reversible reaction. Additionally, the relationship between V_{max} and concentration or between K_m (calculated on V_{max} by Michaelis–Menten equation) and concentration was studied at five different concentrations (0–4 μg/mL), as outlined in Fig. 2(b & c). Apparently, both of V_{max} and K_m decreased with the increase of concentration of **16**, indicating that **16** might be an uncompetitive or mixed-type inhibitor of FBPase.

3.4. In silico docking

In order to further clarify the inhibition modality of our novel series to FBPase, **22**, one of the compounds most potently suppressing hepatocyte glucose output, was selected for a molecular docking analysis using Ligandfit available with Discovery Studio 2.1. Compound **22** may not bind at the FBPase active site since the enzyme was still significantly inhibited even at a high dose of the substrate, namely, there was no obvious competition between compound **22** and the substrate. Instead, the preferred coordination mode of **22** with FBPase should be similar to the mode revealed by Hebeisen [22]. As presented in Fig. 3, although interacts with the

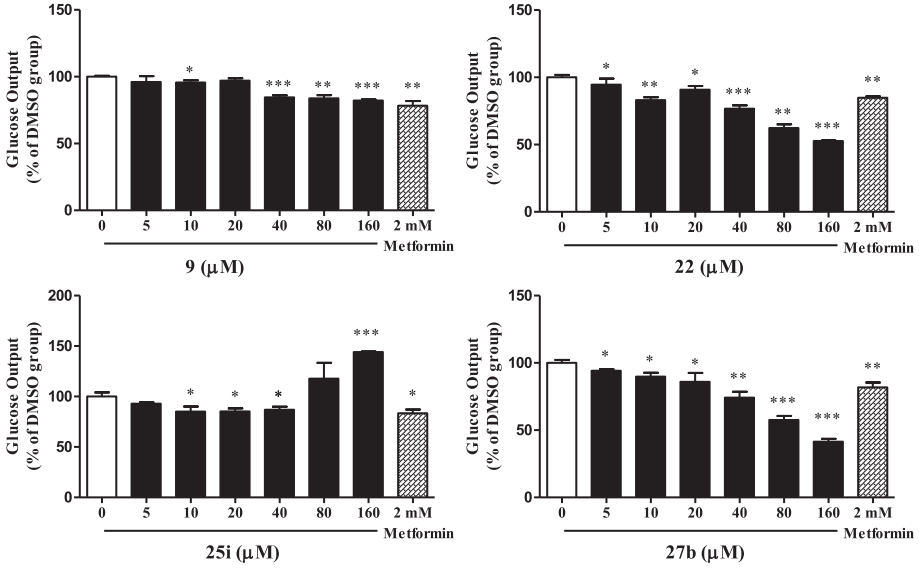


Fig. 1. Inhibition of glucose output in rat hepatocytes by **9**, **22**, **25i** and **27b**; Metformin (2 mM) was used as the control; Significances were expressed by asterisks.

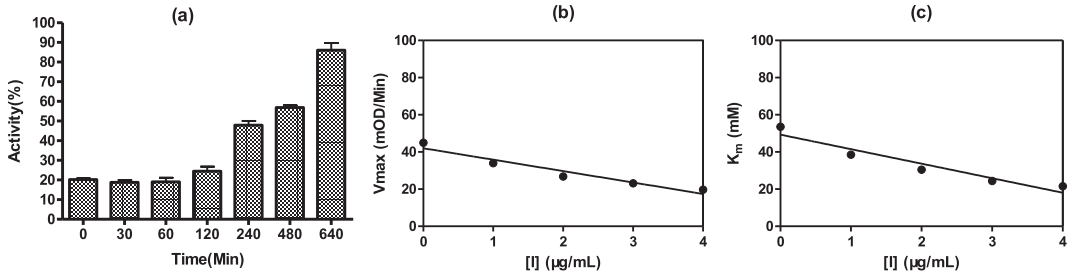


Fig. 2. Kinetic characterization of **16** on FBPase: (a) the reversible binding mode of **16** with enzyme was confirmed by the recovery activity of FBPase after dialysis by indicated times; (b & c) effects on V_{max} and K_m values of FBPase by **16**.

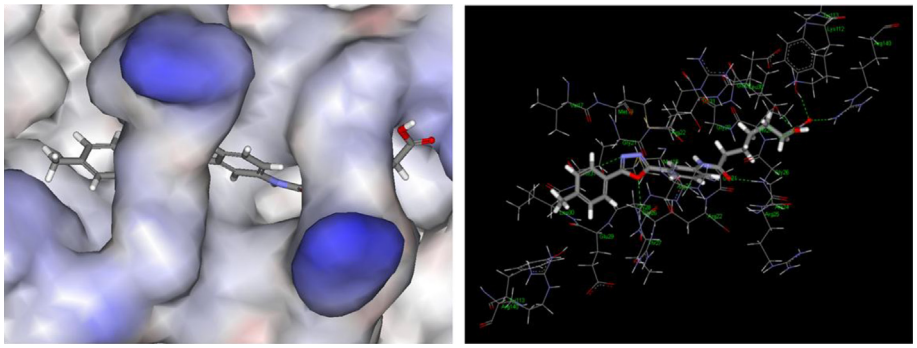


Fig. 3. Compounds **22** docked into FBPase (PDB id = 2JJK). Molecular surface shows hydrophobic regions in white, hydrophilic regions in blue (left graph). Visualization was performed with Discovery Studio 2.1. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

enzyme partly through AMP binding sites, differently, compound **22** binds to two adjacent FBPase monomers simultaneously. The aromatic ring A occupied an area near the AMP purine binding domain on the left monomer through hydrophobic interacts with Leu30 and Tyr113, while at the other end the carboxyl of the ligand sit close to the phosphate recognition pocket (defined by Thr27, Gly28, Glu29, Leu30, Lys112 and Tyr113) of the AMP binding site through multiple hydrogen-bonding interactions with Thr27, Tyr113 and Arg140. The 1,3,4-oxadiazole ring locates at the surface region, engaged in hydrogen bonds with residues Gly28 and Thr31. Aromatic ring B, located at the inter space of the two subunits, acts as a linker without obvious interactions observed, which might imply a possibility of replacement of this ring. The amido bond also binds with Gly26 through a hydrogen bond. It is noted that the distance between the amido and the terminal carboxyl is essential to the binding with residues, by which the SAR of the side chain length would be well explained.

4. Conclusion

In continuing the search for non-phosphorus-based inhibitors of FBPase through targeted structural modifications of previously reported analogues, 2,5-diphenyl-1,3,4-oxadiazoles with a long alkyl side chain, were identified with moderate to good inhibitory activities. In most cases, the tested compounds bearing a terminal hydrogen-bonding acceptor showed potent inhibition against FBPase at molecular level. Moreover, some representative compounds were endowed with cellular activities. The highlighted two compounds **22** and **27b** were able to inhibit the glucose production in primary rat hepatocytes with an IC_{50} value of 167.3 μM and 112.5 μM, respectively. These results indicated that the two compounds are capable of inhibiting GNG likely by interacting with FBPase. In regards to structure activity relationships, an electron-donating group at 4-position on aromatic ring A and a terminal hydroxyl on the side linear alkyl chain are beneficial to the activity at molecular level. However, in consideration of cellular activities, more hydrophilic carboxyl or 1*H*-tetrazole are preferred. Enzymatic kinetic studies of **16** proved the inhibition modality to be reversible mixed-type. In silico docking of **22** with FBPase revealed a possible binding mode, together with enzymatic kinetic properties, which make us to be aware of the suggesting allosteric inhibition to the enzyme. These results may prompt further discovery of promising candidates for new FBPase inhibitors, and thereby treating diabetes.

5. Experimental section

5.1. Biological activity against fructose-1,6-diphosphatase (FBPase)

FBPase recombinant protein was expressed and purified according to the previous report [41]. FBPase activity was measured in a coupled spectrophotometric assay containing 5 mM $MgCl_2$, 66.7 mM KCl, 66.7 mM MOPS pH 7.5, 0.25 mM $NADP^+$, yeast glucose-6-phosphate-dehydrogenase (0.4 U/mL), yeast phosphoglucoisomerase (0.7 U/mL) and 325 nmol/L FBPase. Fructose 6-phosphate formed by the reaction of FBPase was converted to glucose 6-phosphate and subsequently to 6-phosphogluconate by coupling to phosphoglucoisomerase and glucose-6-dehydrogenase and the concomitant formation of NADPH was detected at 340 nm.

5.2. Effect on glucose output of primary hepatocytes

Primary hepatocytes were isolated from Sprague–Dawley rats which was fasted overnight as previously reported [43,44], then suspended in DMEM containing 1 g/L glucose (Invitrogen™) and 10% FBS (Invitrogen™), and plated in 24-well plates (2×10^5 cells in each well). After 4 h' attachment, the old medium was replaced with fresh medium and incubated overnight. The medium was

subsequently replaced with 500 μL glucose and phenol-red-free DMEM supplemented with 20 mmol/L sodium lactate and 2 mmol/L sodium pyruvate, containing 0, 5, 10, 20, 40, 80 and 160 μM relevant compounds with three parallels. After 4 h' incubation, 50 μL medium was collected and the glucose concentration was measured by a colorimetric glucose assay kit (Fudan-Zhangjiang™, Shanghai, China).

5.3. Characterization of the FBPase inhibitors

To characterize the inhibitor of FBPase, the assay was carried out in a 50 μL system containing 5 mM $MgCl_2$, 66.7 mM KCl, 66.7 mM MOPS (pH 7.5), 0.25 mM $NADP^+$, yeast glucose-6-phosphate-dehydrogenase (0.4 U/mL), yeast phosphoglucose isomerase (0.7 U/mL), 325 nmol/L FBPase, FBP(fructose-1,6-bisphosphate) in 2-fold dilution from 400 μmol/L, and different concentrations of the inhibitor, where K_m is the Michaelis constant, v is the initial rate, Vmax is the maximum rate, and [S] is the substrate concentration. The V_{max} and K_m values of the FBPase in the presence and absence of the inhibitor were derived from a non-linear regression fitting of the curve in the plot of the initial rates and the substrate concentrations, using the Michaelis–Menton equation $v = V\max \cdot [S]/(K_m + [S])$.

5.4. Chemistry

Microwave assisted synthesis was performed using a DISCOVER-STM microwave reactor. ^{1}H NMR *spectra*: were recorded at either 400 or 500 MHz on JEOL-400 or Bruker AM-500 instruments respectively. Chemical shifts ($\delta = _H$) are quoted in parts per million (ppm) relative to TMS as internal standard. Signals were characterized as s (singlet), dd (doublet of doublet), t (triplet), m (multiplet), brs (broad signal). ^{13}C NMR *spectra*: were recorded at either 100 or 125 MHz on JEOL-400 or Bruker AM-500 instruments respectively. Chemical shifts ($\delta = _C$) are quoted in ppm relative to TMS as internal standard. Degenerate peaks are suffixed by the number of carbons. *Mass spectra*: High Resolution Mass Spectrometry (HRMS) data were obtained on a Micromass Tof II spectrometer.

5.4.1. 3-Nitrobenzohydrazide (**4**)

To the solution of methyl 3-nitrobenzoate (**3**, 5 g) in dioxane (50 mL) was added hydrazine hydrate aqueoussolution (85%, 10 mL), the mixture was heated to reflux for 6 h, and cooled to room temperature. Evaporation of the solvent in vacuo give a yellow solid, which was then washed with water (5 mL) and dried to afford **4** as a white solid. It was used in next step without any purification.

5.4.2. N'-(4-methylbenzoyl)-3-nitrobenzohydrazide (**6**)

3-Nitrobenzohydrazide (**4**, 10 mmol), *p*-toluicacid (**5**, 10 mmol), EDC (13 mmol) and HOBt (13 mmol) were suspended in anhydrous DCM (25 mL). To this mixture, 3-picoline (14 mmol) was added. After stirring at room temperature for 12 h, the reaction mixture was filtered. The filter cake was washed with 5% HCl and water successively, dried to give **6** as a white solid (yield: 67%). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.37 (s, 3H), 7.32 (d, J = 7.2 Hz, 2H), 7.84–7.86 (m, 3H), 8.36 (d, J = 7.6 Hz, 1H), 8.44 (d, J = 8.0 Hz, 1H), 8.75 (s, 1H), 10.56 (s, 1H), 10.88 (s, 1H); HRMS (ESI): Calcd for $C_{15}H_{14}N_3O_4$ $[M + H]^+$, 300.0979; Found, 300.0976.

5.4.3. 2-(3-Nitrophenyl)-5-p-tolyl-1,3,4-oxadiazole (**7**)

Dihydrazide **6** (10 mmol) was suspended in anhydrous acetonitrile (20 mL). To this mixture phosphorusoxychloride (2 mL) was added. After refluxing for 8 h, the reaction mixture was evaporated in vacuo. The resultant residue was then purified by column chromatography (DCM/hexane = 1/1) to give **7** as a white solid (yield: 86%; mp 164–166 °C). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.46 (s, 3H), 7.36 (d, J = 6.8 Hz, 2H), 7.76 (s, 1H), 8.04 (d, J = 7.3 Hz, 2H), 8.40 (d, J = 7.3 Hz, 1H), 8.50 (d, J = 7.1 Hz, 1H), 8.93 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 165.5, 162.4, 148.7, 142.9, 132.4, 130.4, 129.9(2C), 127.1(2C), 125.9, 125.7, 121.6, 120.6, 21.7; HRMS (ESI): Calcd for $C_{15}H_{12}N_3O_3$ $[M + H]^+$, 282.0873; Found, 282.0869.

5.4.4. 3-(5-p-Tolyl-1,3,4-oxadiazol-2-yl)aniline (**8**)

After the pretreatment by ethanol, Raney-Ni (0.5 g) was suspended in a mixed solution of methanol (20 mL) and THF (20 mL) followed by the addition of **7** (1 g), the mixture was then hydrogenated at 1atm overnight. The catalyst was removed by filtration and the filtrate was evaporated under reduced pressure to give **8**. Yield: 83%; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.36 (s, 3H), 3.82 (s, 2H), 6.77 (s, 1H), 7.19–7.42 (m, 5H), 7.93 (d, J = 7.6 Hz, 2H); HRMS (ESI): Calcd for $C_{15}H_{13}N_3NaO$ $[M + Na]^+$, 274.0956; Found, 274.0999.

5.4.5. N-(3-(5-p-tolyl-1,3,4-oxadiazol-2-yl)phenyl)acetamide(**9**)

To the solution of **8** (2 mmol) and 3-picoline (0.5 mL) in DCM (30 mL) was added acetic anhydride (2.5 mmol) dropwise at 0 °C, then the temperature was raised to 8 °C in 1 h. After stirring at 8 °C for 8 h, aqueous NaOH (5%, 20 mL) was added for hydrolyzing excessive acetic anhydride for 1 h. After that, the DCM phase was separated and washed with water, aqueous HCl (5%, 20 mL) and saturated aqueous $NaHCO_3$ (15 mL) successively. After dried over anhydrous Na_2SO_4, the organic phase was evaporated in vacuo and then purified by column chromatography (DCM/EtOAc = 5/1) to give **9** as a white solid. Yield: 91%; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 2.09 (s, 3H), 2.41 (s, 3H), 7.45 (d, J = 7.8 Hz, 2H), 7.54 (t, J = 8.0 Hz, 1H), 7.77–7.80 (m, 2H), 7.98 (d, J = 7.8 Hz, 2H), 8.40 (s, 1H), 10.24 (s, 1H); HRMS (ESI): Calcd for $C_{17}H_{16}N_3O_2$ $[M + H]^+$, 294.1243; Found, 294.1238.

5.4.6. N-(3-(5-p-tolyl-1,3,4-oxadiazol-2-yl)phenyl)propionamide (**10**)

To the solution of **8** (2 mmol) and 3-picoline (0.5 mL) in DCM (30 mL) was added propionyl chloride (2.5 mmol) dropwise at −5 °C, then the temperature was raised to 5 °C in 1 h. After stirring at 5 °C for 3 h, aqueous NaOH (5%, 20 mL) was added for hydrolyzing excessive propionyl chloride for 1 h. After that, the DCM phase was separated and washed with water, aqueous HCl (5%, 20 mL) and saturated aqueous $NaHCO_3$ (15 mL) successively. After dried over anhydrous Na_2SO_4, the organic phase was evaporated in vacuo and then purified by column chromatography (DCM/EtOAc = 5/1) to give **10** as a white solid. Yield: 77%; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.30 (t, J = 7.6 Hz, 3H), 2.44–2.49 (m, 5H), 7.32(d, J = 8.0 Hz, 2H), 7.47(t, J = 8.0 Hz, 1H), 7.58(t, J = 8.0 Hz, 1H), 7.86 (t, J = 8.0 Hz, 1H), 8.01 (d, J = 8.0 Hz, 2H), 8.26 (s, 1H); HRMS (ESI): Calcd for $C_{18}H_{17}N_3O_2$, $[M + H]^+$, 308.1394; Found, 308.1391.

5.4.7. N-(3-(5-p-tolyl-1,3,4-oxadiazol-2-yl)phenyl)isobutyramide (**11**)

A procedure similar to the preparation of **10** by replacing propionyl chloride with iso-butyryl chloride. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.28 (d, J = 6.9 Hz, 6H), 2.43 (s, 3H), 2.62 (q, J = 6.9 Hz, 1H), 7.30 (d, J = 7.8 Hz, 2H), 7.46 (t, J = 8.0 Hz, 1H), 7.83–7.87 (m, 3H), 8.00 (d, J = 7.8 Hz, 2H), 8.32 (s, 1H); HRMS (ESI): Calcd for $C_{19}H_{20}N_3O_2$, $[M + H]^+$, 322.1556; Found, 322.1551.

5.4.8. N-(3-(5-p-tolyl-1,3,4-oxadiazol-2-yl)phenyl)pentanamide (**12**)

A procedure similar to the preparation of **10** by replacing propionyl chloride with valeryl chloride. ^{1}H NMR (500 MHz, DMSO-d_6): δ = 0.90 (t, J = 6.8 Hz, 3H), 1.30–1.37 (m, 2H), 1.57–1.63 (m, 2H),

2.35 (t, J = 7.5 Hz, 2H), 2.50 (s, 3H), 7.41 (d, J = 7.7 Hz, 2H), 7.53 (t, J = 8.0 Hz, 1H), 7.75 (d, J = 7.5 Hz, 1H), 7.80 (d, J = 7.5 Hz, 1H), 7.96 (d, J = 7.7 Hz, 2H), 8.43 (s, 1H), 10.18 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.8, 164.1, 163.8, 142.1, 140.2, 130.0, 129.9(2C), 126.6(2C), 123.8, 122.1, 121.1, 120.6, 116.6, 36.2, 27.2, 21.9, 21.2, 13.8; HRMS (ESI): Calcd for $C_{20}H_{22}N_3O_2$, $[M + H]^+$, 336.1712; Found, 336.1737.

5.4.9. Methyl 4-oxo-4-(3-(5-p-tolyl-1,3,4-oxadiazol-2-yl) phenylamino)butanoate(**14a**)

In an ice-water bath, to a solution of **8** (1 g, 4 mmol) in THF (15 mL) and Et_3N (2 mL), a solution of succinic anhydride (0.5 g, 5 mmol) in THF (10 mL) was added over 20 min. After stirring at rt overnight, the reaction mixture was poured into ice water (100 mL), followed by adjusting the pH value to 3 by hydrochloric acid. After stirring for 30 min, the precipitate was filtrated and dried to give **14b** as a white solid (0.88 g, yield: 63%, mp 192–195 °C), which was used without any purification.

Anhydrous K_2CO_3 (1.5 g) was added to a solution of **14b** (0.7 g, 2 mmol) and methyl iodide (1 mL) in acetone (15 mL), the resultant mixture was then stirred at room temperature for 20 h. After rotaryevaporation, the residue was dissolved in DCM (30 mL), and then was washed by 5% NaOH、5%HCl and water successively. The separated DCM phase was dried with anhydrous Na_2SO_4, then filtrated and evaporated to give **14a** as a white solid (0.7 g, yield: 99%).^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.43 (s, 3H), 2.77–2.80 (m, 4H), 3.73 (s, 3H), 7.31 (d, J = 8.2 Hz, 2H), 7.45 (t, J = 8.0 Hz, 1H), 7.83–7.85 (m, 2H), 8.00 (d, J = 8.2 Hz, 2H), 8.27 (s, 1H), 8.45 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.9, 165.2, 164.6, 163.0, 142.7, 139.1, 129.7(2C), 129.6, 126.8(2C), 124.2, 122.9, 122.1, 120.8, 117.8, 52.2, 31.7, 29.3, 21.7.

5.4.10. 4-Hydroxy-N-(3-(5-p-tolyl-1,3,4-oxadiazol-2-yl)phenyl) butanamide (**14**)

$NaBH_4$ (0.3 g) was suspended in a solution of **14a** (0.9 g, 2.46 mmol) in THF (20 mL), the mixtured was then heated to reflux. 2 mL of methanol was added to the hot solution dropwise. After 1 h's reflux, the solution was then quenched by diluted hydrochloric acid followed by the evaporation of THF. The resultant residue was dissolved in DCM (30 mL), MeOH (10 mL) and water (50 mL), the organic phase was collected and dried over anhydrous Na_2SO_4, and then purified by column chromatography (DCM/MeOH = 20/1) to give **14** as a white solid (0.35 g, yield: 42%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.75–1.81(m, 2H), 2.40–2.43(m, 5H), 3.46–3.50(m, 2H), 4.40(t, J = 5.1 Hz, 1H), 7.43(d, J = 7.9 Hz, 2H), 7.53(t, J = 7.8 Hz, 1H), 7.76 (d, J = 7.8 Hz, 1H), 7.82 (d, J = 7.7 Hz, 1H), 7.98 (d, J = 7.9 Hz, 2H), 8.43 (s, 1H), 10.08 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 171.6, 164.0, 163.7, 142.1, 140.1, 129.8(2C), 129.7, 126.4(2C), 123.6, 122.1, 120.9, 120.5, 116.6, 60.1, 33.1, 28.2, 21.0; HRMS (ESI): Calcd for $C_{19}H_{20}N_3O_3$ $[M + H]^+$, 338.1499; Found, 338.1437.

5.4.11. Methyl 5-oxo-5-(3-(5-p-tolyl-1,3,4-oxadiazol-2-yl) phenylamino)pentanoate (**15a**)

Compound **15a** was prepared starting from **8** and glutaric anhydride through a procedure similar to that for the preparation of **14a**. White solid. White solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.04–2.11 (m, 2H), 2.39 (s, 3H), 2.45 (t, J = 7.3 Hz, 2H), 2.54 (t, J = 7.3 Hz, 2H), 3.65 (s, 3H), 7.26 (d, J = 8.0 Hz, 2H), 7.41 (t, J = 8.0 Hz, 1H), 7.79 (d, J = 7.8 Hz, 1H), 7.88 (d, J = 7.8 Hz, 1H), 7.93 (d, J = 8.0 Hz, 2H), 8.33 (s, 1H), 8.81 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.6, 171.2, 164.7, 164.1, 142.3, 139.1, 129.7(2C), 129.6, 126.8(2C), 124.2, 123.0, 122.1, 120.7, 117.9, 51.5, 36.1, 33.0, 29.5, 21.5.

5.4.12. 5-Hydroxy-N-(3-(5-p-tolyl-1,3,4-oxadiazol-2-yl)phenyl) pentanamide (**15**)

Compound **15** was prepared starting from **15a** through a procedure similar to that for the preparation of **14**. White solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.47(m, 2H), 1.65(m, 2H), 2.35 (t, J = 7.3 Hz, 2H), 2.37 (s, 3H), 3.43(m, 2H), 4.42(t, J = 5.2 Hz, 1H), 7.40 (d, J = 8.0 Hz, 2H), 7.51 (t, J = 7.8 Hz, 1H), 7.73 (d, J = 7.8 Hz, 1H), 7.80 (d, J = 7.8 Hz, 1H), 7.94 (d, J = 8.0 Hz, 2H), 8.42 (s, 1H), 10.16 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 171.7, 164.0, 163.7, 142.1, 140.2, 129.9(2C), 129.8, 126.5(2C), 123.7, 122.1, 121.0, 120.5, 116.6, 60.4, 36.3, 32.0, 21.7, 21.1; HRMS (ESI): Calcd for $C_{20}H_{22}N_3O_3$ $[M + H]^+$, 352.1656; Found, 352.1699.

5.4.13. 6-Hydroxy-N-(3-(5-p-tolyl-1,3,4-oxadiazol-2-yl)phenyl) hexanamide (**16**)

In an ice-water bath, to a mixture of **8** (0.25 g,1 mmol), EDC (0.4 g), HOBt (0.3 g) and new-preprared 6-hydroxyhexanoic acid (0.13 g) in DCM (10 mL) was added 3-picoline (0.3 mL), and after stirring for 24 h, the reaction solution was diluted by DCM (20 mL) and methanol (8 mL). The organic solution was washed by 5% HCl, 5% NaOH and saturated $NaHCO_3$ successively, dried over anhydrous Na_2SO_4, and then purified by column chromatography (DCM/EtOAc = 3/1) to give **16** as a white solid (0.21 g, yield: 57%). mp 128–130 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 1.31–1.36 (m, 2H), 1.41–1.46 (m, 2H), 1.59–1.62 (m, 2H), 2.31–2.35 (m, 5H), 3.39–3.41(m, 2H), 4.36(t, J = 5.2 Hz, 1H), 7.36 (d, J = 7.9 Hz, 2H), 7.49 (t, J = 7.8 Hz, 1H), 7.71 (d, J = 7.8 Hz, 1H), 7.79 (d, J = 7.8 Hz, 1H), 7.91 (d, J = 7.9 Hz, 2H), 8.40 (s, 1H), 10.13(s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6): δ = 171.7, 164.0, 163.7, 142.1, 140.2, 129.9(2C), 129.8, 126.4(2C), 123.7, 122.0, 120.9, 120.5, 116.6, 60.6, 36.5, 32.3, 25.2, 25.0, 21.0; HRMS (ESI): Calcd for $C_{21}H_{24}N_3O_3$ $[M + H]^+$, 366.1812; Found, 366.1833.

5.4.14. 7-Hydroxy-N-(3-(5-p-tolyl-1,3,4-oxadiazol-2-yl)phenyl) heptanamide (**17**)

In an ice-water bath, to a mixture of **8** (0.25 g, 1 mmol), EDC (0.4 g), HOBt (0.3 g) and monomethyl heptanedioate (**17b**, 0.15 g) in DCM (10 mL) was added 3-picoline (0.3 mL), and after stirring for 24 h, the reaction solution was diluted by DCM (20 mL) and methanol (8 mL). The organic solution was washed by 5% HCl, 5% NaOH and saturated $NaHCO_3$ successively, dried over anhydrous Na_2SO_4, and then purified by column chromatography (DCM/EtOAc = 10/1) to give **17a** as a white solid (0.25 g, yield: 61%).

17a was reduced by $NaBH_4$ by a procedure similar to that for the preparation of **14** to give **17** as a white solid. mp 125–128 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 1.23–1.27(m, 2H), 1.30–1.35(m, 2H), 1.60–1.63 (m, 2H), 2.34(t, J = 7.3 Hz, 2H), 2.39 (s, 3H), 3.39 (t, J = 6.4 Hz, 2H), 7.41 (d, J = 7.8 Hz, 2H), 3.52 (brs, 1H), 7.52 (t, J = 7.7 Hz, 1H), 7.75 (d, J = 7.7 Hz, 1H), 7.80 (d, J = 7.7 Hz, 1H), 7.96 (d, J = 7.8 Hz, 2H), 8.43 (s, 1H), 10.15(s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6): δ = 171.7, 164.0, 163.7, 142.2, 140.2, 129.9(2C), 129.8, 126.5(2C), 123.7, 122.1, 121.0, 120.6, 116.6, 60.7, 36.4, 32.4, 28.6, 25.3, 25.0, 21.1; HRMS (ESI): Calcd for $C_{22}H_{26}N_3O_3$ $[M + H]^+$, 380.1969; Found, 380.1933.

5.4.15. 8-Hydroxy-N-(3-(5-p-tolyl-1,3,4-oxadiazol-2-yl)phenyl) octanamide (**18**)

18 was prepared by a procedure similar to that for the preparation of **17**, starting from **8** and monomethyl suberate(**18b**). ^{1}H NMR (500 MHz, DMSO-d_6): δ = 1.23–1.25 (m, 6H), 1.39–1.42 (m, 2H), 1.60–1.62 (m, 2H), 2.34 (t, J = 7.3 Hz, 2H), 2.40 (s, 3H), 3.35–3.37 (m, 2H), 4.37 (t, J = 5.3 Hz, 1H), 7.43 (d, J = 7.8 Hz, 2H), 7.53 (t, J = 7.7 Hz, 1H), 7.76 (d, J = 7.7 Hz, 1H), 7.80 (d, J = 7.7 Hz, 1H), 7.97 (d, J = 7.8 Hz, 2H), 8.44 (s, 1H), 10.19(s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6): δ = 171.8, 164.1, 163.8, 142.2, 140.2, 130.0(2C), 129.9,

126.6(2C), 123.7, 122.1, 121.1, 120.6, 116.6, 60.7, 36.5, 32.5, 28.7, 28.7, 25.4, 25.0, 21.2; HRMS (ESI): Calcd for $C_{23}H_{28}N_3O_3$ $[M + H]^+$, 394.2125; Found, 394.2185.

5.4.16. 9-Hydroxy-N-(3-(5-p-tolyl-1,3,4-oxadiazol-2-yl)phenyl) nonanamide (**19**)

19 was prepared by a procedure similar to that for the preparation of **17**, starting from **8** and monomethyl azelate (**19b**). mp 111–113 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 1.23–1.28(m, 8H), 1.38–1.40(m, 2H), 1.59–1.62(m, 2H), 2.34 (t, J = 7.3 Hz, 2H), 2.40(s, 3H), 3.35–3.37(m, 2H), 4.34(brs, 1H), 7.43 (d, J = 7.8 Hz, 2H), 7.53 (t, J = 8.0 Hz, 1H), 7.76 (d, J = 7.7 Hz, 1H), 7.80 (d, J = 8.0 Hz, 1H), 7.97 (d, J = 7.8 Hz, 2H),8.43 (s, 1H), 10.17 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6): δ = 171.8, 164.1, 163.8, 142.2, 140.2, 130.0(2C), 129.9, 126.6(2C), 123.8, 122.2, 121.2, 120.6, 116.6, 60.7, 36.5, 32.5, 28.9, 28.9, 28.7, 25.5, 25.0, 21.2; HRMS (ESI): Calcd for $C_{24}H_{30}N_3O_3$ $[M + H]^+$, 408.2282; Found,408.2228.

5.4.17. 10-Hydroxy-N-(3-(5-p-tolyl-1,3,4-oxadiazol-2-yl)phenyl) decanamide (**20**)

20 was prepared by a procedure similar to that for the preparation of **17**, starting from **8** and monomethyl sebacate (**20b**). mp 111–113 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 1.23–1.28 (m, 10H), 1.37–1.39(m, 2H), 1.59–1.61 (m, 2H), 2.32 (t, J = 7.3 Hz, 2H), 2.41 (s, 3H), 3.35–3.37 (m, 2H), 4.31(t, J = 5.3 Hz, 1H), 7.44 (d, J = 7.8 Hz, 2H), 7.53 (t, J = 7.7 Hz, 1H), 7.77 (d, J = 7.7 Hz, 1H), 7.80 (d, J = 7.7 Hz, 1H), 7.98 (d, J = 7.8 Hz, 2H), 8.43 (s, 1H), 10.17(s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6): δ = 171.3, 164.1, 163.80, 142.3, 140.2, 130.0(2C), 129.9, 126.6(2C), 123.8, 122.2, 121.1, 120.6, 116.6, 60.7, 36.5, 32.5, 29.0, 28.9, 28.8, 28.7, 25.5, 25.0, 21.2; HRMS (ESI): Calcd for $C_{25}H_{32}N_3O_3$ $[M + H]^+$, 422.2438; Found, 422.2477.

5.4.18. N-(3-(5-p-tolyl-1,3,4-oxadiazol-2-yl)phenyl)hex-5-enamide (**21a**)

21a was prepared by a procedure similar to that for the preparation of **17a**, starting from **8** and hex-5-enoic acid (**21b**). White solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.83–1.89 (m, 2H), 2.13 (d, J = 6.6 Hz, 2H), 2.39–2.46 (m, 5H), 4.96–5.04 (m, 2H), 5.73–5.83 (m, 1H), 7. 29 (d, J = 7.9 Hz, 2H), 7.43 (t, J = 7.8 Hz, 1H), 7.81 (d, J = 7.8 Hz, 1H), 7.81 (d, J = 7.8 Hz, 1H), 7. 97 (d, J = 7.9 Hz, 2H), 8.34 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 171.9, 164.8, 164.1, 142.4, 139.0, 137.7, 137.6, 129.7(2C), 126.9(2C), 124.3, 123.1, 122.3, 120.8, 118.0, 115.4, 36.7, 33.0, 24.5, 21.6.

5.4.19. 5,6-Dihydroxy-N-(3-(5-p-tolyl-1,3,4-oxadiazol-2-yl)phenyl) hexanamide (**21**)

K_3 $[Fe(CN)_6]$ (0.7 g), K_2CO_3 (0.3 g) and $K_2OsO_2(OH)_4$ (0.05 g) were dissolved in the solution composed of tertiarybutanol (3 mL) and water (3 mL) at 0 °C, followed by the adding of **21a** (0.2 g,0.57 mmol). After reacting at 0 °C for 5 h, the reaction mixture stirred at rt for 12 h. Sodium hydrosulfite (1 g) was added to the mixture before the inner temperature was reduced to 0 °C, and after stirring at rt for 1 h, 20 mL water was added. The resultant precipitation was filtered and dissolved in a mixed solution of DCM and MeOH(1/1), after filtration, the filtrate was evaporated and triturated with ether to give **21** as a white solid (0.19 g, yield: 84%). mp 149–151 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 1.26–1.28 (m, 1H), 1.47–1.49 (m, 1H), 1.61–1.63 (m, 1H), 1.75–1.77 (m, 1H), 2.34 (t, J = 7.4 Hz, 2H), 2.35 (s, 3H), 3.24–3.29 (m, 2H), 3.41–3.44 (m, 1H), 4.45–4.49 (m, 2H), 7.38 (d, J = 7.8 Hz, 2H), 7.50 (t, J = 7.7 Hz, 1H), 7.75 (d, J = 7.7 Hz, 1H), 7.79(d, J = 7.7 Hz, 1H), 7.93 (d, J = 7.8 Hz, 2H), 8.41 (s, 1H), 10.16(s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6): δ = 171.8, 164.0, 163.7, 142.1, 140.2, 129.9(2C), 129.8, 126.5(2C), 123.7, 122.1, 121.0, 120.5, 116.6, 70.9, 65.9, 36.7, 32.9, 21.5, 21.1; HRMS (ESI): Calcd for $C_{21}H_{23}N_3NaO_4$ $[M + Na]^+$, 404.1581; Found, 404.1510.

5.4.20. 6-Oxo-6-(3-(5-p-tolyl-1,3,4-oxadiazol-2-yl)phenylamino) hexanoic acid (**22**)

In an ice-water bath, to a mixture of **8** (0.25 g, 1 mmol), pimelic acid (1.6 g), EDC (0.3 g) and HOBt (0.25 g) in DCM (20 mL) was added 3-picoline (0.3 mL), and after stirring for 24 h, the reaction solution was flash washed by 5% HCl. Vigorous stirring of the collected DCM phase with 20mLwater precipitated mass of white solid. The following filtration and trituration with ether gave **22** (70 mg, yield: 18%). ^{1}H NMR (500 MHz, DMSO-d_6): δ = 1.44–1.48 (m, 2H), 1.63–1.78 (m, 4H), 2.35–2.64 (m, 7H), 7.52 (d, J = 8.0 Hz, 2H), 7.65 (t, J = 7.9 Hz, 1H), 7.84–7.94 (m, 2H), 8.07 (d, J = 8.0 Hz, 2H), 8.55 (s, 1H), 10.30 (s, 1H), 12.17 (brs,1H); ^{13}C NMR (125 MHz, DMSO-d_6): δ = 174.5, 171.7, 164.0, 163.8, 142.2, 140.2, 130.0 (2C), 129.9, 126.6 (2C), 123.8, 122.1, 121.1, 120.6, 116.7, 36.3, 33.6, 28.2, 24.8, 24.3, 21.2; Calcd for $C_{22}H_{22}N_3O_4$ $[M-H]^+$, 392.1610; Found, 392.1637.

5.4.21. 3-Chloro-N-(3-(5-p-tolyl-1,3,4-oxadiazol-2-yl)phenyl) propanamide (**23a**)

In an ice-water bath, to a solution of **8** (1 g, 3.98 mmol) and 3-picoline (1 mL) in DCM (20 mL) was added 4-chlorobutyryl chloride (0.7 mL), then let the solution raise to rt naturally. After stirring overnight, the mixture was diluted with DCM (25 mL) and washed with 5% HCl, 5% NaOH and saturated $NaHCO_3$ successively, dried over anhydrous Na_2SO_4, and then purified by column chromatography (DCM/EA = 10/1) to give **23a** as a white solid (0.87 g, yield: 57%). mp 106–108 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.21–2.26 (m, 2H), 2.35 (s, 3H), 2.63 (t, J = 7.1 Hz, 2H), 3.69 (t, J = 6.1 Hz, 2H), 7.33 (d, J = 8.0 Hz, 2H), 7.49 (t, J = 8.0 Hz, 1H), 7.61 (s,1H), 7.82 (d, J = 8.0 Hz, 1H), 7.88 (d, J = 8.0 Hz, 1H), 8.02 (d, J = 8.0 Hz, 2H), 8.27 (s,1H).

5.4.22. 3-Iodo-N-(3-(5-p-tolyl-1,3,4-oxadiazol-2-yl)phenyl) propanamide (**24a**)

The mixture of **23a** (0.8 g,2 mmol), NaI (4.5 g, 28 mmol) and anhydrous acetone (30 mL) was heated to reflux, after stirring for 12 h the reaction mixture was cooled and evaporated. The resultant residue was dissolved in DCM (30 mL) and water (50 mL), and then the organic phase was separated, dried over anhydrous Na_2SO_4, filtrated, and then evaporated to give **24a** as an off-white solid (containing 9.5% **23a**), which was used in the next step without purification. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.22–2.28 (m, 2H), 2.35 (s, 3H), 2.59 (t, J = 7.0 Hz, 2H), 3.31 (t, J = 6.6 Hz, 2H), 7.32 (d, J = 8.0 Hz, 2H), 7.48 (t, J = 8.0 Hz, 1H), 7.84–7.87 (m, 2H), 7.93 (s,1H), 8.01 (d, J = 8.0 Hz, 2H), 8.31 (s,1H).

5.4.23. 4-Morpholino-N-(3-(5-p-tolyl-1,3,4-oxadiazol-2-yl)phenyl) butanamide (**25a**)

To the solution of **24a** (0.2 g, 0.4 mmol) in THF (10 mL) was added morpholine (4 mmol), and then let the solution stir overnight. After evaporation in vacuo, the resultant residue was dissolved in a solution composed of DCM (25 mL) and methanol (5 mL), washed by 5% NaOH and water, dried over anhydrous Na_2SO_4 and then purified by column chromatography (DCM/MeOH = 10/1) to give **25a** as a white solid (87 mg, yield: 50%). ^{1}H NMR (500 MHz, DMSO-d_6): δ = 1.75–1.80 (m, 2H), 2.31–2.42 (m, 11H), 3.54–3.56 (m, 4H), 7.45 (d, J = 8.0 Hz, 2H), 7.54 (t, J = 7.6 Hz, 1H), 7.77 (d, J = 7.6 Hz, 1H), 7.81 (d, J = 8.0 Hz, 2H), 7.99 (d, J = 8.0 Hz, 2H), 8.45 (s, 1H), 10.18 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6): δ = 171.7, 164.4, 164.1, 142.7, 140.3, 130.3(2C), 130.2, 126.9(2C), 123.9, 122.5, 121.5, 120.7, 117.0, 66.6(2C), 57.9, 53.4(2C), 34.7, 22.0, 21.4; HRMS (ESI): Calcd for $C_{23}H_{27}N_4O_3$ $[M + H]^+$, 407.2078; Found, 407.2033.

*5.4.24. 4-(Piperidin-1-yl)-N-(3-(5-p-tolyl-1,3,4-oxadiazol-2-yl) phenyl)butanamide (**25b**)*

25b was prepared by a procedure similar to that for the synthesis of **25a** with the replacement of mopholine with piperidine. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.21–1.29 (m, 2H), 1.55–1.59 (m, 2H), 1.78–1.81 (m, 4H), 2.00–2.05 (m, 2H), 2.45(s, 3H), 2.61–2.66 (m, 8H), 7.33 (d, J = 7.9 Hz, 2H), 7.47 (t, J = 7.6 Hz, 1H), 7.86–7.88 (m, 2H), 8.02 (d, J = 7.9 Hz, 2H), 8.33 (s, 1H), 9.94 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 171.6, 164.8, 164.2, 142.3, 139.4, 129.7(2C), 129.6, 126.9(2C), 124.4, 123.0, 122.2, 121.1, 117.9, 57.4, 54.1(2c), 35.6, 29.7, 24.9(2C), 23.7, 21.6, 21.5; HRMS (ESI): Calcd for $C_{25}H_{31}N_4O_2$ $[M + H]^+$, 419.2447; Found, 419.2479.

*5.4.25. 5-Morpholino-N-(3-(5-p-tolyl-1,3,4-oxadiazol-2-yl)phenyl) pentanamide (**25c**)*

25c was prepared by a procedure similar to that for the synthesis of **25b** with the replacement of **24a** with **24b**. White solid; mp 140–142 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 1.46–1.51 (m, 2H), 1.57–1.62 (m, 2H), 2.33–2.44 (m, 11H), 3.56 (t, J = 4.4 Hz, 4H), 7.40 (d, J = 8.0 Hz, 2H), 7.50 (t, J = 7.6 Hz, 1H), 7.73 (s, 1H), 7.78 (d, J = 7.6 Hz, 1H), 7.93 (d, J = 8.0 Hz, 2H), 8.42 (s, 1H), 10.21 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6): δ = 171.6, 164.0, 163.7, 142.2, 140.1, 130.0(2C), 129.9, 126.6(2C), 123.7, 122.1, 121.1, 120.5, 116.6, 65.6 (2C), 57.0, 52.9(2C), 36.1, 24.9, 22.7, 21.1; HRMS (ESI): Calcd for $C_{24}H_{29}N_4O_3$ $[M + H]^+$, 421.2234; Found, 421.2265.

*5.4.26. 5-(4-Methylpiperazin-1-yl)-N-(3-(5-p-tolyl-1,3,4-oxadiazol-2-yl)phenyl)pentanamide (**25d**)*

25d was prepared by a procedure similar to that for the synthesis of **25c** with the replacement of mopholine with N-methyl piperazine. White solid; mp 182–184 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 1.45–1.49 (m, 2H), 1.58–1.63 (m, 2H), 2.16 (s, 3H), 2.28–2.40 (m, 15H), 7.44 (d, J = 8.0 Hz, 2H), 7.53 (t, J = 7.6 Hz, 1H), 7.77 (d, J = 7.6 Hz, 1H), 7.81 (d, J = 7.6 Hz, 1H), 7.98 (d, J = 8.0 Hz, 2H), 8.44 (s, 1H), 10.21 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6): δ = 171.7, 164.1, 163.8, 142.3, 140.2, 130.0(2C), 129.9, 126.6(2C), 123.7, 122.1, 121.1, 120.6, 116.6, 57.4, 54.5(2C), 52.4(2C), 45.5, 36.2, 25.8, 22.9, 21.1; HRMS (ESI): Calcd for $C_{25}H_{32}N_5O_2$ $[M + H]^+$, 434.2551; Found, 434.2558.

*5.4.27. 5-(Piperazin-1-yl)-N-(3-(5-p-tolyl-1,3,4-oxadiazol-2-yl) phenyl)pentanamide (**25e**)*

25e was prepared by a procedure similar to that for the synthesis of **25d** with the replacement of N-methyl piperazine with piperazine. White solid; ^{1}H NMR (500 MHz, DMSO-d_6):δ = 1.45–1.47 (m, 2H), 2.25–2.41 (m, 13H), 2.65–2.67 (m, 2H), 7.44 (d, J = 8.0 Hz, 2H), 7.54 (t, J = 7.6 Hz, 1H), 7.77 (d, J = 7.6 Hz, 1H), 7.81 (d, J = 7.6 Hz, 1H), 8.00 (d, J = 8.0 Hz, 2H), 8.44 (s, 1H), 10.19 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6): δ = 171.7, 164.0, 163.7, 142.2, 140.1, 130.0(2C), 129.9, 126.5(2C), 122.1, 120.6, 116.6, 107.9, 58.3, 54.2(2C), 45.6(2C), 36.3, 25.6, 23.0, 21.1; HRMS (ESI): Calcd for $C_{24}H_{30}N_5O_2$ $[M + H]^+$, 420.2394; Found, 420.2371.

*5.4.28. 5-(4-Hydroxypiperidin-1-yl)-N-(3-(5-p-tolyl-1,3,4-oxadiazol-2-yl)phenyl)pentanamide (**25f**)*

25f was prepared by a procedure similar to that for the synthesis of **25d** with the replacement of N-methyl piperazine with 4-hydroxypiperidine. White solid; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 1.44–1.78 (m, 10H), 2.37–2.42 (m, 7H), 2.83–2.88 (m, 2H), 3.52–3.57 (m,1H), 4.70 (brs, 1H), 7.46 (d, J = 8.0 Hz, 2H), 7.55 (t, J = 7.6 Hz, 1H), 7.79 (d, J = 7.6 Hz, 1H), 7.82 (d, J = 7.6 Hz, 1H), 8.00(d, J = 8.0 Hz, 2H), 8.47 (s, 1H), 10.29 (s, 1H); HRMS (ESI): Calcd for $C_{25}H_{31}N_4O_3$ $[M + H]^+$, 435.2391; Found, 435.2425.

*5.4.29. 5-(4-Acetylpiperazin-1-yl)-N-(3-(5-p-tolyl-1,3,4-oxadiazol-2-yl)phenyl)pentanamide (**25g**)*

In an ice-water bath, **25e** (50 mg) was suspended in DCM (5 mL), then to the mixture was added triethylamine (40 mg) and acetic anhydride (40 mg). After raising to rt, the reaction was allowed to stir overnight. By evaporating and then recrystallizing in ethanol, a white solid (21 mg) was obtained. mp 160–162 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 1.49–1.51 (m, 2H), 1.62–1.64 (m, 2H), 1.97 (s, 3H), 2.37–2.40 (m, 11H), 3.40–3.42 (m, 4H), 7.43 (d, J = 6.7 Hz, 2H), 7.53 (t, J = 7.8 Hz, 1H), 7.76 (d, J = 7.1 Hz, 1H), 7.82 (d, J = 7.9 Hz, 1H), 7.97 (d, J = 6.8 Hz, 2H), 8.45 (s, 1H), 10.23 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6): δ = 171.6, 168.1, 164.0, 163.7, 142.2, 140.1, 130.0(2C), 129.9, 126.5(2C), 123.7, 122.1, 121.0, 120.5, 116.6, 57.2, 52.8 (2C), 52.3(2C), 40.5, 36.1, 25.4, 22.8, 21.1; HRMS (ESI): Calcd for $C_{26}H_{32}N_5O_3$ $[M + H]^+$, 462.2500; Found, 462.2542.

*5.4.30. 6-(4-Methylpiperazin-1-yl)-N-(3-(5-p-tolyl-1,3,4-oxadiazol-2-yl)phenyl)hexanamide (**25h**)*

25h was prepared by a procedure similar to that for the synthesis of **25d** with the replacement of **24c** with **24b**. White solid. mp 132–134 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 1.27–1.30 (m, 2H), 1.40–1.43 (m, 2H), 1.59–1.62 (m, 2H), 2.11 (s, 3H), 2.21–2.39 (m, 15H), 7.41 (d, J = 8.0 Hz, 2H), 7.52 (t, J = 7.6 Hz, 1H), 7.75 (d, J = 7.6 Hz, 1H), 7.80 (d, J = 7.6 Hz, 1H), 7.96 (d, J = 8.0 Hz, 2H), 8.43 (s, 1H), 10.21 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6): δ = 171.7, 164.0, 163.8, 142.2, 140.2, 130.0(2C), 129.9, 126.6(2C), 123.7, 122.1, 121.0, 120.6, 116.6, 57.7, 54.6(2C), 52.6(2C), 45.6, 36.4, 26.5, 26.1, 25.0, 21.1; HRMS (ESI): Calcd for $C_{26}H_{34}N_5O_2$ $[M + H]^+$, 448.2707; Found, 448.2747.

*5.4.31. 6-Morpholino-N-(3-(5-p-tolyl-1,3,4-oxadiazol-2-yl)phenyl) hexanamide (**25i**)*

25i was prepared by a procedure similar to that for the synthesis of **25h** with the replacement of N-methyl piperazine with morpholine. White solid; mp 135–137 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 1.30–1.33 (m, 2H), 1.41–1.46 (m, 2H), 1.59–1.64 (m, 2H), 2.24 (t, J = 7.3 Hz, 2H), 2.30–2.36 (m, 6H), 2.41 (s, 3H), 3.53 (t, J = 4.5 Hz, 4H), 7.44 (d, J = 8.0 Hz, 2H), 7.53 (t, J = 7.6 Hz, 1H), 7.77 (d, J = 7.6 Hz, 1H), 7.80 (d, J = 7.6 Hz, 1H), 7.98 (d, J = 8.0 Hz, 2H), 8.44 (s, 1H), 10.19 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6): δ = 171.7, 164.1, 163.8, 142.3, 140.2, 130.0(2C), 129.9, 126.6(2C), 123.7, 122.1, 121.1, 120.6, 116.6, 66.2(2C), 58.2, 53.4(2C), 36.4, 26.5, 25.7, 25.0, 21.2; HRMS (ESI): Calcd for $C_{25}H_{31}N_4O_3$ $[M + H]^+$, 435.2391; Found, 435.2394.

*5.4.32. 2-(4-(5-Oxo-5-(3-(5-p-tolyl-1,3,4-oxadiazol-2-yl) phenylamino)pentyl)piperazin-1-yl)acetic acid (**26b**)*

tert-Butyl bromoacetate (2.6 g) was added dropwise to a solution of piperazine (4 g) in EtOH (40 mL) at room temperature. After stirring overnight, the solution was evaporated in vacuo with a hot water bath (no higher than 40 °C). After 30 mL ethanol was distilled off, the residue was poured into 50 mL of water and extracted with ether (20 mL). The separated ether phase was washed by saturated brine, dried over anhydrous Na_2SO_4, and then evaporated to afford **28** as a yellow oil (1.8 g).

To the solution of **24b** (0.2 g, 0.4 mmol) in THF (8 mL) and acetonitrile (8 mL) was added **28** (70 mg) and K_2CO_3 (0.2 g). After stirring for 36 h at rt, DCM (10 mL) and methanol (5 mL) was added to dilute the reacting mixture, then filtrate the mixture. The obtained filtration was evaporated and purified by column chromatography (DCM/EtOAc/MeOH = 25/5/1) to give **26a** (0.17 g), containing a small amount of **28** but able to use without any further purification.

In an ice-water bath, a solution of **26a** (0.17 g) in DCM (15 mL) was added dropwise to the mixture of TFA (5 mL) and DCM (5 mL) over 10 min. After raising to rt naturally, the reaction was stirred for

4 h and then evaporated. Dissolving the resultant residue in 20 mL water followed by the adjusting of pH to 7 with 10% HCl resulted in a mass of precipitation. After filtrating, the obtained solid was then recrystallized in ethanol to give **26b** (90 mg) as a white solid. mp 166–167 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.42–1.44 (m, 2H), 1.57–1.60 (m, 2H), 2.25–2.50 (m, 15H), 2.83 (s, 2H), 7.40 (d, J = 7.2 Hz, 2H), 7.49 (t, J = 7.4 Hz, 1H), 7.71 (d, J = 7.4 Hz, 1H), 7.79 (d, J = 7.4 Hz, 1H), 7.94 (d, J = 7.2 Hz, 2H), 8.41 (s,1H), 10.24 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 171.6, 171.6, 164.0, 163.7, 142.1, 140.1, 129.9(2C), 129.7, 126.5(2C), 123.6, 122.0, 120.9, 120.5, 116.6, 62.5, 57.5, 52.7(2C), 52.3(2C), 36.2, 25.9, 23.0, 21.1; HRMS (ESI): Calcd for $C_{26}H_{30}N_5O_4$ $[M-H]^+$, 476.2298; Found, 476.2271.

5.4.33. 5-Cyano-N-(3-(5-p-tolyl-1,3,4-oxadiazol-2-yl)phenyl) pentanamide (**27a**)

To a mixture of **24b** (0.22 g) with acetonitrile (8 mL) was added TMSCN (90 mg) and TBAF (0.25 g) successively at room temperature. After stirring overnight, the reacting solution was evaporated and purified by column chromatography (DCM/EA = 5/1) to afford **27a** as a light yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.67–1.71 (m, 2H), 1.88–1.94 (m, 2H), 2.43–2.46 (m, 5H), 2.64 (t, J = 6.9 Hz, 2H), 7.31 (d, J = 7.9 Hz, 2H), 7.44 (t, J = 7.8 Hz, 1H), 7.82 (d, J = 7.8 Hz, 1H), 7.97 (d, J = 7.8 Hz, 1H), 8.01 (d, J = 7.9 Hz, 2H), 8.52 (s, 1H), 8.98 (s, 1H).

5.4.34. 5-(1H-tetrazol-5-yl)-N-(3-(5-p-tolyl-1,3,4-oxadiazol-2-yl) phenyl)pentanamide (**27b**)

27a (0.12 g), $TMSN_3$ (0.5 mL) and TBAF (0.3 g) was dissolved in NMP (4 mL), then the solution was reacted by the microwave irradiation under 120 °C for 30 min. After evaporated in vacuo, the resultant residue was purified by column chromatography (DCM/MeOH = 10/1) to give a light yellow oil, which was then triturated with DCM to give **27b** as a white solid (62 mg, yield: 59%). mp 170–171 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.66–1.71 (m, 2H), 1.77–1.81 (m, 2H), 2.40–2.42 (m, 5H), 2.94 (t, J = 7.2 Hz, 2H), 7.45 (d, J = 7.8 Hz, 2H), 7.55 (t, J = 7.9 Hz, 1H), 7.77–7.83 (m, 2H), 7.99 (d, J = 7.8 Hz, 2H), 8.45 (s, 1H), 10.21 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 171.3, 164.0, 163.7, 155.8, 142.2, 140.0, 130.0(2C), 129.9, 126.5(2C), 123.7, 122.1, 121.1, 120.6, 116.6, 35.8, 26.6, 24.3, 22.5, 21.1. HRMS (ESI): Calcd for $C_{21}H_{22}N_7O_2$ $[M+H]^+$, 404.1835; Found, 404.1865.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.06.011.

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