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

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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].

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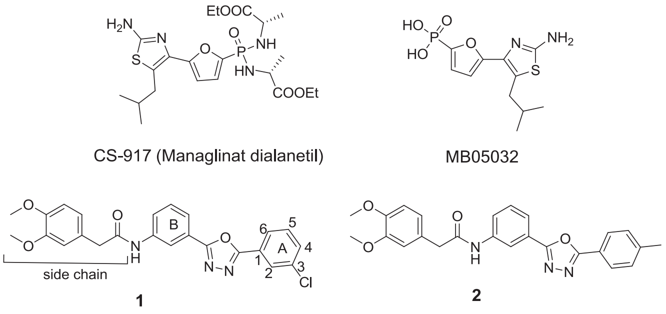

Chart 1. The previously reported inhibitors of FBPase.

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 ($IC_{50} = 1.3 \pm 0.44$ 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 ($IC_{50} = 7.70 \pm 0.34$ 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

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