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Synthesis, SAR, and X-ray structure of tricyclic compounds as potent FBPase inhibitors

Tomoharu Tsukada [a], Mizuki Takahashi [b], Toshiyasu Takemoto [a], Osamu Kanno [a], Takahiro Yamane [a], Sayako Kawamura [b], Takahide Nishi [a,*]

[a] Medicinal Chemistry Research Laboratories I, Daiichi Sankyo Co., Ltd, 1-2-58 Hiromachi, Shinagawa-ku, Tokyo 140-8710, Japan
[b] Exploratory Research Laboratories I, Daiichi Sankyo Co., Ltd, 1-16-13 Kitakasai, Edogawa-ku, Tokyo 134-8630, Japan

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ABSTRACT

With the aim of discovering a novel class of fructose-1,6-bisphosphatase (FBPase) inhibitors, a series of compounds based on tricyclic scaffolds was synthesized. Extensive SAR studies led to the finding of **8l** with an IC_{50} value of 0.013 μM against human FBPase. An X-ray crystallographic study revealed that **8l** bound at AMP binding sites of human liver FBPase with hydrogen bonding interactions similar to AMP.

Type 2 diabetes mellitus (T2DM), which accounts for more than 90% of all diabetes, is characterized by high levels of glucose in the plasma. The global incidence of this disease is predicted to rise to more than 366 million by the year 2030.[1] T2DM usually leads to complications such as retinopathy, nephropathy, and neuropathy. Clinical studies have suggested that fasting hyperglycemia in T2DM is associated with excessive glucose production through gluconeogenesis.[2] Thus, the inhibition of gluconeogenesis is a potentially useful approach to reducing the increased blood glucose levels in patients with T2DM.

Fructose-1,6-bisphosphatase (FBPase), which is predominantly expressed in the liver and the kidney and catalyzes the hydrolysis of fructose-1,6-bisphosphate (F1,6BP) to fructose-6-phosphate (F6P), is one of the rate-limiting enzymes of gluconeogenesis.[3] FBPase is a homotetramer and exists in two distinct conformational states, an active R-state and an inactive T-state. F1,6BP and F6P in combination with metal cations stabilize the active R-state, while adenosine 5′-monophosphate (AMP, **1**) and substrate analogue fructose-2,6-bisphosphate (F2,6BP) synergistically stabilize the inactive T-state.[4] AMP and/or F2,6BP are natural inhibitors of FBPase which bind to the allosteric site and the substrate-binding site, respectively, and control blood glucose levels by regulating the activity of FBPase. FBPase inhibitors would lower blood glucose levels by reducing hepatic glucose output and are expected to be a novel class of drugs for the treatment of T2DM.

Several classes of small-molecule inhibitors of FBPase have been reported. These inhibitors can be structurally classified into two groups; non-phosphorus-based inhibitors and phosphorus-based inhibitors. In the former group, several chemotypes including anilinoquinazoline,[5] indole dicarboxylic acid,[6] piperazinedione,[7] and benzoxazole benzenesulfonamide[8] were reported, although they showed only modest inhibitory potency against FBPase. Also in this group, bissulfonylurea[9] was described as a potent FBPase inhibitor with novel interaction. In the latter group, in contrast, AMP mimetic MB05032 (**2**) exhibited high inhibitory activity. A prodrug of MB05032 (CS-917, **3**) lowered blood glucose levels in animal models and was entered into clinical development (Fig. 1).[10]

With the intention of developing a novel class of FBPase inhibitors, we turned our attention to phosphorus-based AMP mimetics in recognition of their in vitro and in vivo potencies. From the binding mode of AMP and FBPase, the hydrogen bonding interactions of the NH_2 and PO_3H_2 groups of AMP seemed to play an important role in realizing high affinity.[4a] Based on this structural information, we focused our attention on placing the two groups at the appropriate position to obtain such high affinity. AMP mimetic MB05032 has a rotatable biheteroaryl moiety; we expected that increasing the structural rigidity of the compound would be useful to fix the NH_2 and PO_3H_2 groups in the appropriate binding orientation. This led us to design rigid tricyclic scaffolds, and the three kinds of the scaffolds were designed by varying the central ring size from a five- to a seven-membered ring (Fig. 2). In order to facilitate changing the positions of the PO_3H_2 group, a benzene ring instead of a furan ring was introduced to the tricyclic scaffolds.

* Corresponding author. Tel.: +81 3 3492 3131; fax: +81 3 5436 8563.
E-mail address: nishi.takahide.xw@daiichisankyo.co.jp (T. Nishi).

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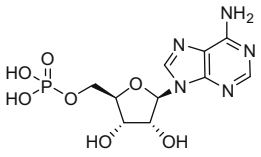
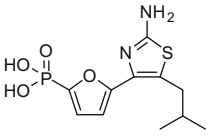
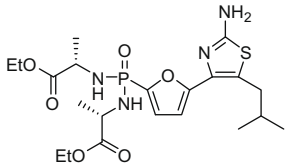


1 (AMP)

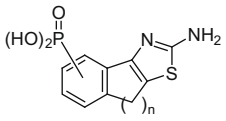


2 (MB05032)



3 (CS-917)

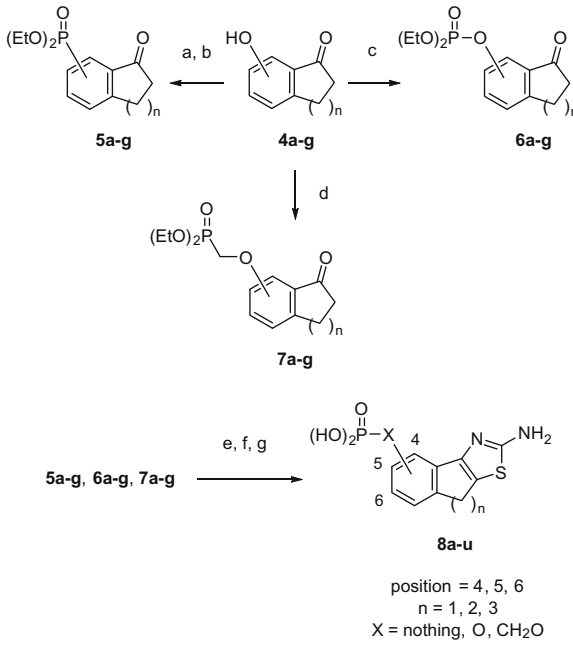
Figure 1. Structures of AMP and known FBPase inhibitors.



n = 1, 2, 3

Figure 2. General structure of new FBPase inhibitors based on tricyclic scaffolds.

A series of tricyclic derivatives was efficiently synthesized according to Scheme 1. The bicyclic phenols **4a–g** were converted to diethyl phosphonate **5a–g** via triflation and Pd-catalyzed phosphonylation. Diethyl phosphate **6a–g** were obtained by direct phosphorylation of **4a–g**, and diethyl phosphonate **7a–g** were acquired by condensation of **4a–g** with diethoxyphosphorylmethyl tosylate.[11] Bromination of **5–7** followed by cyclization with thiourea and subsequent hydrolysis afforded the final compounds **8a–u**.

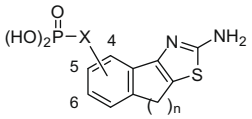


Scheme 1. Synthesis of compounds **8a–u**. Reagents and conditions: (a) 2,6-lutidine, 4-dimethylaminopyridine, Tf_2O, CH_2Cl_2, 46–97%; (b) $Pd(PPh_3)_4$, $(EtO)_2P(O)H$, Et_3N, DMF, 60 °C, 91–98%; (c) $(EtO)_2P(O)H$, Et_3N, CCl_4, 87–95%; (d) $(EtO)_2P(O)CH_2OTs$, K_2CO_3, DMF, 80 °C, 28–72%; (e) $PhNMe_3Br_3$, CH_2Cl_2; (f) thiourea, NaOAc, EtOH, reflux, 14–57% over two steps; (g) TMSBr, CH_2Cl_2, 26–91%.

The inhibitory activities against human FBPase of the tricyclic derivatives are summarized in Table 1. Initially, to optimize the position of the PO_3H_2 group, a variety of benzene phosphonic acid derivatives were prepared (**8a–g**). Most of these compounds showed low activity, but only **8g**, possessing a relatively flexible scaffold with a seven-membered central ring, showed moderate activity (IC_{50} = 0.169 μM). This result suggested that some degree of structural flexibility was beneficial in improving inhibitory activity and prompted us to introduce a spacer group of 1–2 atoms in length connecting the PO_3H_2 group with the tricyclic scaffolds. Due to synthetic feasibility, linkers such as –O– and $–CH_2O–$ were selected for further investigation (**8h–u**). Introducing the spacer groups showed a tendency to increase activity, and several compounds bearing –O– linker (i.e., phosphate compounds) exhibited enhanced activity (**8j**, **8l**, and **8n**, IC_{50} = 0.070, 0.013, and 0.022 μM, respectively). In particular, **8l** exhibited the highest potency, almost equal to that of MB05032.

In order to obtain insights into the binding mode of this novel series of tricyclic inhibitors, an X-ray crystal structure of human liver FBPase in complex with **8l** was determined (Fig. 3).[12] As expected, **8l** binds at four AMP binding sites of FBPase tetramer and the **8l**-bound FBPase exists in the inactive T-state. The phosphate group of **8l** occupies a similar position to that of the phosphate group of AMP. Similarly, the amino group of **8l** is placed in the same position as the $6NH_2$ of AMP. The phosphate group interacts with the backbone nitrogens (NH) of Gly26, Thr27, Gly28, Glu29, Leu30, Thr31 and the side chains of Thr27, Lys112, Tyr113 and Arg140, and some of these interactions are formed via water molecules. A water molecule placed between phosphate and the aromatic N atom also contributes to the hydrogen-bonding network among the phosphate group and FBPase. The amino group of **8l** makes hydrogen bonds with the carbonyl oxygen of Val17

Table 1
FBPase inhibitory activity of compounds **8a–u**



Compound	Position	*n*	X	IC_{50}[a] (μM)
MB05032	—	—	—	0.010
8a	6	1	Nothing	91.9
8b	5	1	Nothing	18.7
8c	4	1	Nothing	28.4
8d	6	2	Nothing	354
8e	5	2	Nothing	9.32
8f	4	2	Nothing	354
8g	5	3	Nothing	0.169
8h	6	1	O	146
8i	5	1	O	307
8j	4	1	O	0.070
8k	6	2	O	112
8l	5	2	O	0.013
8m	4	2	O	335
8n	5	3	O	0.022
8o	6	1	CH_2O	112
8p	5	1	CH_2O	112
8q	4	1	CH_2O	0.124
8r	6	2	CH_2O	16.4
8s	5	2	CH_2O	19.1
8t	4	2	CH_2O	21.5
8u	5	3	CH_2O	41.7

[a] Inhibition of human liver FBPase.

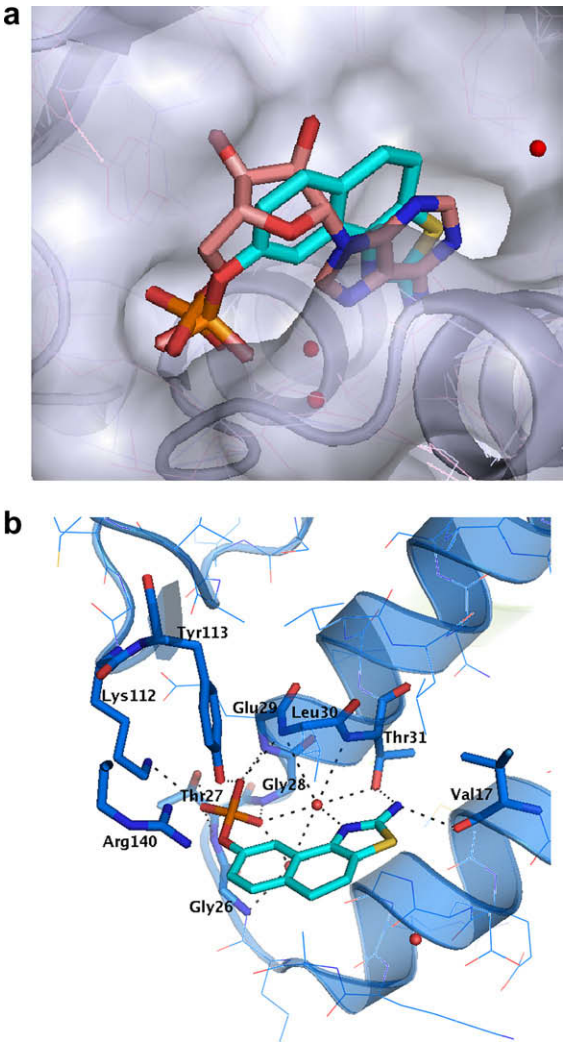
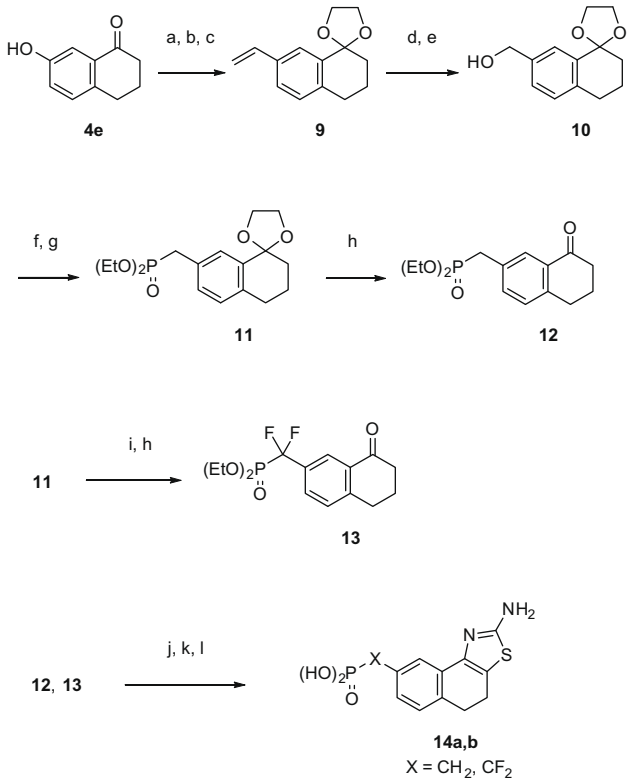


Figure 3. X-ray crystal structure of human liver FBPase in complex with **8l**. (a) Overlay of **8l** and AMP. (b) Details of the interaction of **8l** with FBPase. The residues that interact with **8l** are shown as a stick model.



Scheme 2. Synthesis of compounds **14a**,**b**. Reagents and conditions: (a) 2,6-lutidine, 4-dimethylaminopyridine, Tf_2O, CH_2Cl_2, 98%; (b) ethylene glycol, TsOH, toluene, reflux, 76%; (c) $PdCl_2$(dppf), vinylmagnesium bromide, THF; (d) K_2OsO_4, NMO, $NaHCO_3$, KIO_4, ^tBuOH, THF, H_2O; (e) $NaBH_4$, MeOH, 57% over three steps; (f) PPh_3, CBr_4, CH_2Cl_2, 0 °C, 84%; (g) $(EtO)_3P$, 150 °C, 51%; (h) PPTS, acetone, H_2O, reflux, 85–88%; (i) NaHMDS, *N*-fluorobenzenesulfonimide, THF, −78 °C, 80%; (j) $PhNMe_3Br_3$, CH_2Cl_2; (k) thiourea, NaOAc, EtOH, reflux, 44–64% over two steps; (l) TMSBr, CH_2Cl_2, 81–93%.

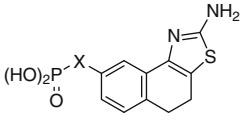
(Val17CO) and Thr31Oγ. These hydrogen bonds may contribute to causing the quaternary conformational change of FBPase towards the inactive T-state.[4b,c] This complex structure suggests that the tricyclic scaffold of **8l** is beneficial in retaining the proper distance and directions between the phosphate group and the amino group. In addition, this planar tricyclic scaffold can fit well to the pocket of the AMP-binding site, resulting in relatively high affinity and therefore high inhibitory activity of **8l**.

Although **8l** exhibited high inhibitory activity, **8l** was found to be metabolically unstable due to the hydrolysis of the phosphate group (data not shown), which prompted us to modify the phosphate moiety of **8l**. Comparison of the activities between three compounds bearing no or different spacer groups (**8e**, **8l**, and **8s**, IC_{50} = 9.32, 0.013, and 19.1 μM, respectively) and the X-ray structure of **8l** bound to FBPase suggested that a spacer group of 1 atom in length was favorable for potent inhibitory activity. Therefore, we decided to evaluate phosphate mimics such as methylenephosphonate and difluoromethylenephosphonate.

The syntheses of these phosphate mimics are illustrated in Scheme 2. Methylene analog **14a** and difluoromethylene analog **14b** were prepared from 7-hydroxy-1-tetralone **4e**. Triflation of **4e** followed by ketalization and Pd-catalyzed coupling with vinyl bromide gave olefin **9**. Olefin **9** was transformed into alcohol **10** through dihydroxylation, oxidative cleavage, and subsequent reduction. Diethyl phosphonate **11** was obtained by bromination of **10** and subsequent Arbuzov reaction. Deketalization of **11** provided ketone **12**, and difluorination of **11** followed by deketalization led to ketone **13**. Bromination of **12**, **13** followed by cyclization with thiourea and subsequent hydrolysis afforded the final compounds **14a,b**.

The inhibitory activities against human FBPase of these analogs are summarized in Table 2. Methylene analog **14a** displayed reduced activity (IC_{50} = 0.840 μM) relative to **8l**. In contrast, difluoromethylene analog **14b** showed similar potency (IC_{50} = 0.047 μM) to **8l**. Interestingly, the second pK_a of benzylic α,α-difluorophosphonic acid (5.71) is more like that of phenylphosphate (6.22) than that

Table 2
FBPase inhibitory activity of compounds **14a,b**



Compound	X	IC_{50} (μM)[a]
8l	O	0.013
14a	CH_2	0.840
14b	CF_2	0.047

[a] Inhibition of human liver FBPase.

of benzylic phosphonic acid (7.72),[13] which suggests that the high charge density on the PO_3H_2 group is favorable to the enzyme-inhibitor interaction. Contrary to the phenyl phosphate structure in **8l**, the difluoromethylenephosphonate structure in **14b** is chemically stable to hydrolysis. This finding provided us with the possibility for further development of this tricyclic scaffold as an FBPase inhibitor.

In summary, we designed and synthesized a series of tricyclic compounds as FBPase inhibitors. Compound **8l** exhibited high inhibitory activity, and an X-ray crystallographic study revealed that **8l** binds in the AMP binding site with hydrogen bonding interactions similar to those of AMP. In order to enhance metabolic stability, the structure of **8l** was modified to difluoromethylene analog **14b** which showed inhibitory activity similar to **8l**. Further studies on enhancing the inhibitory activity and developing prodrug compounds are being intensively conducted at this time.

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12. The X-ray crystallographic study was accomplished according to the procedure described in Ref. 4a. Crystals of FBPase-**8l** complex were grown using the hanging drop vapor diffusion method at 22 °C from a protein solution (6–10 mg/mL FBPase, 20 mM Tris/HCl (pH 8.5), 1 mM DTT, 0.1 mM EDTA, 1 mM **8l**) combined with an equal volume of a reservoir solution (8–10% PEG3350, 0.15 M NaCl, and 0.1 M Tris/HCl (pH 8.5)). The FBPase-**8l** cocrystal was diffracted to 2.6 Å in resolution and the structure was solved by the molecular replacement method with a tetramer model of the published human FBPase structure (PDB 1fta). The coordinate and statistics of FBPase-**8l** complex are available from the PDB using accession code 3a29.
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