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Discovery of potent and orally active tricyclic-based FBPase inhibitors

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ABSTRACT

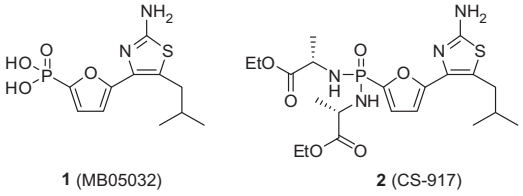
With the aim of exploring the effect of tricyclic-based FBPase inhibitors in cells and in vivo, a series of prodrugs of tricyclic phosphonates was designed and synthesized. Introducing prodrug moieties into tricyclic-based phosphonates led to the discovery of prodrug **15c**, which strongly inhibited glucose production in monkey hepatocytes. Furthermore, prodrug **15c** lowered blood glucose levels in fasted cynomolgus monkeys.

1. Introduction

Type 2 diabetes mellitus (T2DM), which accounts for more than 90% of all diabetics, is characterized by fasting hyperglycemia and an excessive rise in the plasma glucose concentration after glucose or meal ingestion. 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 useful approach in reducing increased blood glucose levels in patients with T2DM.

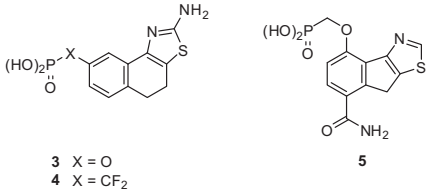
Fructose-1,6-bisphosphatase (FBPase) is one of the rate-limiting enzymes of hepatic gluconeogenesis.[3] FBPase is predominantly expressed in the liver and the kidney and catalyzes the hydrolysis of fructose-1,6-bisphosphate to fructose-6-phosphate. FBPase inhibitors would lower blood glucose levels by reducing hepatic glucose output and are expected to be a novel class of drugs for the treatment of T2DM. There are several small-molecule inhibitors of FBPase,[4–9] in which AMP mimetic MB05032 (**1**) exhibited high inhibitory activity (Fig. 1). The prodrug of MB05032 (CS-917, **2**) lowered blood glucose levels in animal models and was in clinical development.[10]

In the previous paper, we described the design and synthesis of tricyclic thiazoles as FBPase inhibitors, and a series of SAR studies led to the identification of phosphate **3** and difluoromethylene-phosphonate **4** exhibiting potent FBPase inhibitory activities (IC_{50} = 13, 47 nM, respectively) (Fig. 2).[11] In addition, in order to improve metabolic stability and enhance FBPase inhibitory activity, we further developed tricyclic-based FBPase inhibitors with the aid of



1 (MB05032) **2** (CS-917)

Figure 1. Structures of known FBPase inhibitors.



3 X = O
4 X = CF_2

5

Figure 2. Structures of tricyclic-based FBPase inhibitors.

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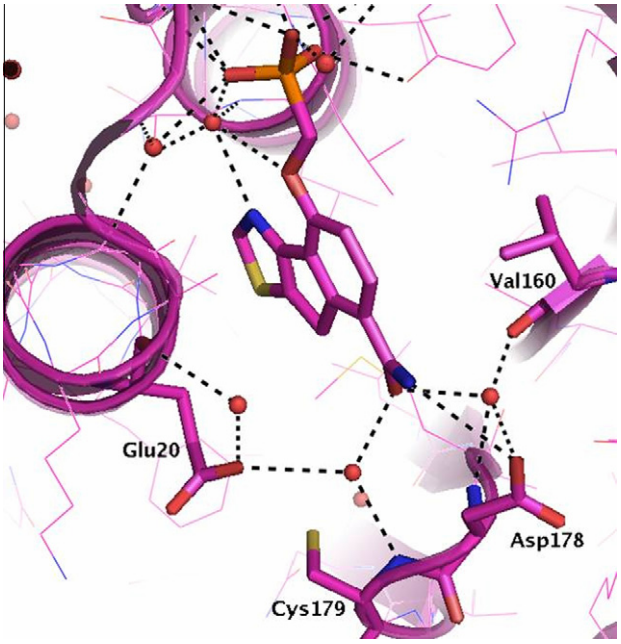
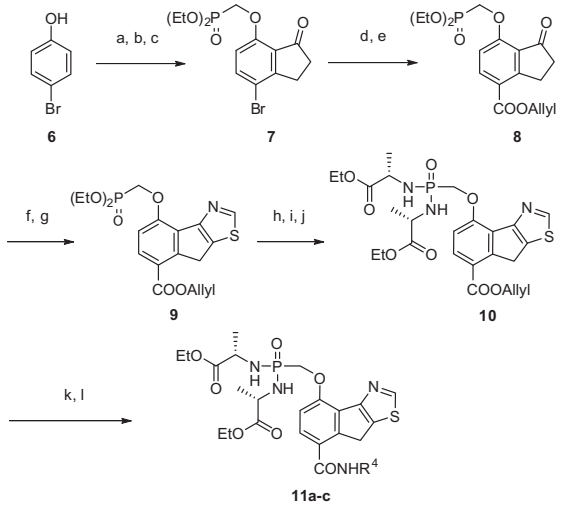


Figure 3. X-ray crystal structures of human liver FBPase in complex with **5**.



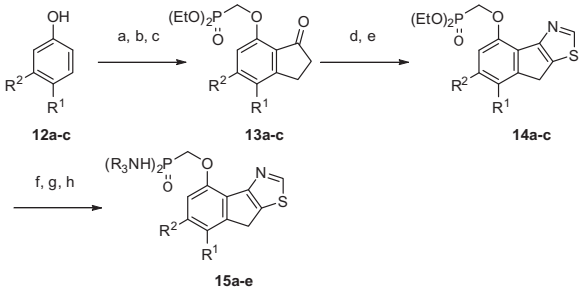
Scheme 1. Synthesis of prodrugs **11a**–**c**. Reagents and conditions: (a) $ClCH_2CH_2COCl$, pyridine, CH_2Cl_2, 0 °C to rt, 85%; (b) $AlCl_3$, 180 °C, 36%; (c) $(EtO)_2P(O)CH_2OTs$, K_2CO_3, DMF, 80 °C, 67%; (d) $Pd(OAc)_2$, dppp, CO, Et_3N, $TMSCH_2CH_2OH$, DMF, 70 °C, 55%; (e) TBAF, allyl bromide, Et_3N, 93%; (f) $CuBr_2$, EtOH, 60 °C, 91%; (g) P_2S_5, $HCONH_2$, THF, reflux, 71%; (h) TMSBr, CH_2Cl_2; (i) $(COCl)_2$, DMF, CH_2Cl_2; (j) Ala-OEt, DIPEA, CH_2Cl_2, −20 °C to rt, 69% over three steps; (k) $Pd(PPh_3)_4$, PPh_3, pyrrolidine, MeCN, 50 °C, 92%; (l) amine, DIPEA, WSC, HOBt, DMF, 56–88%.

structure-based drug design, which led to the discovery of phosphonate **5**, exhibiting more potent FBPase inhibitory activity (IC_{50} = 1 nM).[12] The key feature of phosphonate **5** was that this compound possessed no amino group which might be the cause of metabolic instability. The X-ray co-crystal structure of **5** suggested that high affinity was achieved by hydrophobic interaction which compensated for the loss of the amino group, and also by a hydrogen-bonding network involving the amide side chain of **5** (Fig. 3).

This paper describes our continuing efforts to explore the effect of the tricyclic phosphonates in cells and in vivo. Our phosphonate compounds seemed to have low membrane permeability due to the high negative charge of phosphonate moieties, which prompted us to convert the phosphonate compounds into corresponding prodrugs. There are many classes of phosphonate prodrugs,[13] from which we selected phosphonic diamides as prodrugs in consideration of the advantage that the cleavage byproducts are nontoxic amino acids. In addition, the significant glucose-lowering effect of diamide prodrug CS-917 in animal models encouraged us to explore this type of prodrug. In order to investigate the cellular activity and in vivo efficacy of our tricyclic-based inhibitors, we focused our attention on developing diamide prodrugs of tricyclic phosphonates.

2. Results and discussion

The prodrugs of the tricyclic phosphonates were synthesized according to Schemes 1 and 2. The prodrugs containing amide side chains (**11a**–**c**) were prepared from commercially available 4-bromophenol **6** (Scheme 1). Acylation of **6** followed by a Fries rearrangement and the introduction of a diethyl phosphonate unit resulted in diethyl phosphonate **7**. CO insertion of **7** followed by a transesterification reaction afforded allyl ester **8**, which was transformed into tricyclic thiazole **9** via bromination and cyclization with thioformamide (in situ generation). L-Alanine diamide **10** was obtained by cleaving the phosphonate ethyl groups of **9**, dichlorination, and subsequent condensation with L-alanine ethyl esters. Cleaving an allyl group of **10** and amidation with corresponding amines led to prodrugs **11a**–**c**. Prodrugs containing alkyl side chains were synthesized by methods similar to those described for prodrugs **11a**–**c**, using side chains containing phenols

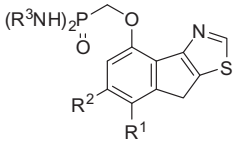


Scheme 2. Synthesis of prodrugs **15a**–**e**. Reagents and conditions: (a) $ClCH_2CH_2COCl$, pyridine, CH_2Cl_2, 0 °C to rt, 56–72%; (b) $AlCl_3$, 180 °C, 32–90%; (c) $(EtO)_2P(O)CH_2OTs$, K_2CO_3, DMF, 80 °C, 78–98%; (d) $CuBr_2$, EtOH, 60 °C, 90–99%; (e) P_2S_5, $HCONH_2$, THF, reflux, 57–66%; (f) TMSBr, CH_2Cl_2; (g) $(COCl)_2$, DMF, CH_2Cl_2; (h) amino acid ester, DIPEA, CH_2Cl_2, −20 °C to rt, 55–65% over three steps.

12a–**c** as starting materials (Scheme 2). The prodrugs **15a**–**e** were obtained by condensation with the corresponding amino acid esters in the final step.

The inhibitory effects on glucose production in monkey hepatocytes varied widely with the side chains of the parent phosphonates (Table 1). With the intent to rigorously evaluate the in vivo potential of prodrugs, this cell assay was performed under severe conditions wherein the hepatocytes were preincubated with prodrugs for only a short time (2 min). Initially, phosphonate **5**, which possesses an amide side chain and demonstrated potent inhibitory activities against human and monkey FBPase (IC_{50} = 1, 22 nM, respectively), was converted to L-alanine diamide **11a**. However, prodrug **11a** showed only a modest inhibitory effect on glucose production, about ninefold less potent than CS-917. Similarly, prodrug **11b** and **11c** containing another amide side chain showed little effect. In contrast, our efforts to convert the phosphonates containing alkyl side chains to prodrugs resulted in a major increase in inhibitory activities in the cell assays. Prodrug **15a** containing a methyl side chain exhibited high inhibitory activity (IC_{50} = 7.4 μM), whereas prodrug **15b** containing a ethyl side chain

Table 1
The inhibitory effects on glucose production in hepatocytes



Compound	R^1	R^2	R^3	Human FBPase IC_{50}[a] (nM)	Monkey FBPase IC_{50}[b] (nM)	Hepatocytes IC_{50}[c] (μM)
11a	$CONH_2$	H	(*S*)-CH(Me)CO_2Et	1	22	94
11b	CONHMe	H	(*S*)-CH(Me)CO_2Et	2	19	>100
11c	$CONHCH_2{}^tBu$	H	(*S*)-CH(Me)CO_2Et	11	31	>100
15a	Me	H	(*S*)-CH(Me)CO_2Et	8	24	7.4
15b	Et	H	(*S*)-CH(Me)CO_2Et	11	23	23
15c	Me	Me	(*S*)-CH(Me)CO_2Et	10	34	6.8
15d	Me	Me	(*S*)-CH(Me)$CO_2{}^iPr$	10	34	9.0
15e	Me	Me	CH_2CO_2Et	10	34	9.4
CS-917	—	—	—	10	30	10

[a] Inhibition of human liver FBPase (corresponding parent phosphonates).
[b] Inhibition of monkey liver FBPase (corresponding parent phosphonates).
[c] Inhibition of glucose production in primary monkey hepatocytes.

was about threefold less potent (IC_{50} = 23 μM) than **15a**. In addition, prodrug **15c** containing two methyl side chains exhibited inhibitory activity (IC_{50} = 6.8 μM) similar to that of prodrug **15a**. Prodrugs derived from other amino acid esters such as L-alanine isopropyl esters (**15d**) and glycine ethyl esters (**15e**) were slightly less potent (IC_{50} = 9.0, 9.4 μM, respectively) than L-alanine diamide **15c**. Large differences in the inhibitory activity between prodrugs **11a–c** and **15a–e** suggested that a judicious combination of tricyclic-based phosphonate and prodrug moiety was required to exhibit high inhibitory activity in cell assays.

The physicochemical properties of the prodrugs provided important clues for understanding a broad range of inhibitory activities in the cell assays (Table 2). Some physicochemical properties are useful in predicting drug absorption. Considering Lipinski's rules[14] and the polar surface area (PSA),[15] L-alanine diamide prodrugs **11a–c** and **15a–c** appear to have different physicochemical parameters with respect to *c*Log *P* and PSA. In particular, compared to prodrugs **15a–c**, which possess alkyl side chains and exhibited potent inhibitory effects in the cell assays, prodrugs **11a–c** have high PSA values due to the presence of amide side chains. PSA is defined as the sum of the surfaces of the polar atoms in a molecule and correlate well with cell membrane permeability. A short preincubation of hepatocytes with prodrugs in the cell assays resulted in placing emphasis on the cell membrane permeability of the prodrugs. It was suggested that prodrugs **15a–c** exhibited high inhibitory activities in the cell assays, presumably due to low PSA values and relatively high membrane permeability.

Prodrug **15c** exhibiting high inhibitory activity in the cell assays also demonstrated in vivo efficacy in an animal model (Fig. 4). In order to investigate the in vivo efficacy of the prodrugs, fasted cynomolgus monkeys were used as an animal model, considering that the fasting plasma glucose level correlates with the hepatic glucose production originating in gluconeogenesis. Prodrug **15c** was administered orally at 20 mg/kg to fasted cynomolgus monkeys. The blood glucose levels of the monkeys were significantly decreased 4 h after dosing compared to the vehicle-treated animals. Hypoglycemia, a safety concern of gluconeogenesis inhibition, was not observed. In addition, the plasma concentration of prodrug **15c** and corresponding phosphonate **16** in this in vivo study showed that prodrug **15c** disappeared rapidly and the corre-

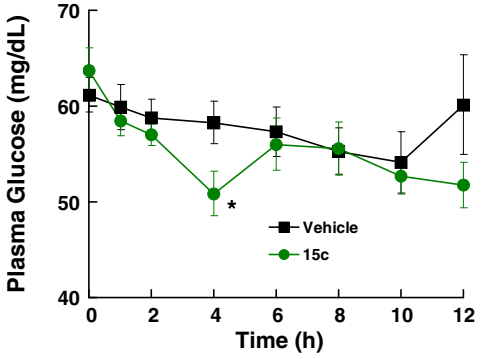


Figure 4. Oral glucose-lowering effect of prodrug **15c** in fasted cynomolgus monkeys (20 mg/kg). Data were the mean ± S.E.M (n = 7, $^*P$ <0.05 compared to vehicle group, paired *t*-test).

Table 2
Physicochemical properties of L-alanine diamide prodrugs[a]

Compound	MW	HB-D[b]	HB-A[c]	*c*Log *P*	PSA[d]	Hepatocytes IC_{50}[e] (μM)
11a	524.53	4	11	0.69	196.99	94
11b	538.55	3	11	0.9	183	>100
11c	594.66	3	11	2.66	183	>100
15a	495.53	2	9	2.47	153.9	7.4
15b	509.56	2	9	3	153.9	23
15c	509.56	2	9	2.93	153.9	6.8

[a] Calculated using ACD/labs version 9.0 (Advanced Chemistry Development, Inc.).
[b] Hydrogen bond donors.
[c] Hydrogen bond acceptors.
[d] Polar surface area ($Å^2$).
[e] Inhibition of glucose production in primary monkey hepatocytes (experimental values).

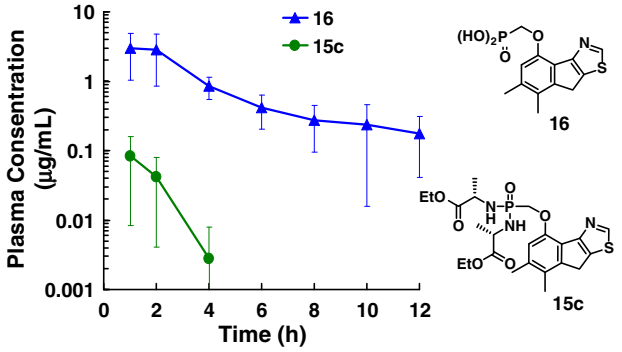


Figure 5. Plasma concentration of prodrug **15c** and corresponding parent phosphonate **16** in cynomolgus monkeys. Data were the mean ± SD (n = 7).

sponding parent phosphonate **16** remained for a long time at relatively high concentrations (Fig. 5). This result suggested that prodrug **15c** was rapidly converted to corresponding phosphonate **16** in the body and L-alanine diamide prodrug moiety functioned as a prodrug. Reliable evidence of the in vivo efficacy of prodrug **15c** has provided the possibility of developing new drugs for the treatment of T2DM.

3. Conclusion

In summary, we developed prodrugs of tricyclic-based FBPase inhibitors in order to investigate their effects in cells and in vivo. In contrast to prodrugs containing amide side chains, prodrugs containing alkyl side chains potently inhibited glucose production in monkey hepatocytes. The physicochemical properties of prodrugs suggested that low PSA values and relatively high membrane permeability was probably critical to the exhibition of high inhibitory activity in the cell assays. The in vivo study in fasted cynomolgus monkeys revealed that prodrug **15c** lowered blood glucose levels and was rapidly converted to corresponding phosphonate **16**. These results have provided the possibility of developing new drugs for the treatment of T2DM.

4. Experimental

4.1. Biological assays

4.1.1. Gluconeogenesis inhibition in monkey hepatocytes

Primary monkey hepatocytes (KAC, Kyoto, Japan) were incubated overnight in DMEM containing 5% FBS, 1 μM dexamethasone, and 100 μM dbcAMP before being washed with Dulbecco's phosphate buffered saline containing 25 mM sodium bicarbonate, 25 mM HEPES and 1% BSA (test medium). The hepatocytes were preincubated in the test medium with test compounds for 2 min, followed by subsequent incubation for 4 h with gluconeogenic substrates (10 mM lactate and 1 mM pyruvate) after washing out the test compounds. The glucose concentration in the medium was measured by Glucose CII-test Wako (Wako Pure Chemical Industries, Ltd, Osaka, Japan).

4.1.2. In vivo efficacy in cynomolgus monkeys

Vehicle (50% PEG 400, 0.5% MC, 0.5% Tween 80) or prodrug **15c** at 20 mg/kg (dissolved in the vehicle) was orally administered to overnight-fasted cynomolgus monkeys (n = 7, 5–8 years old). The same monkeys were used for each regimen after a washout period of at least 2 weeks. Blood samples were collected just before dosing and 1, 2, 4, 6, 8, 10, and 12 h after dosing. The plasma glucose levels in each sample were measured using an i-STAT 300F blood analyzer (i-STAT Corp., Princeton, NJ). The concentrations of prodrug **15c** and phosphonate **16** were also measured by using LC–MS/MS analysis (Quattro micro, Micromass Ltd).

4.2. Chemistry

NMR spectra were recorded on a Varian Mercury 400 or 500 spectrometer with tetramethylsilane as an internal reference. Infrared spectra were recorded on a Jasco FT/IR-830 spectrophotometer. Mass spectra were recorded on a JEOL JMS-AX505H. TLC analysis was performed on $60F_{254}$ plates. Column chromatography was performed on Silica gel 60 (Merck, 230–400).

4.2.1. Diethyl {[(6,7-dimethyl-3-oxo-2,3-dihydro-1*H*-inden-4-yl)oxy]methyl}phosphonate (13c)

A solution of 3,4-dimethylphenol **12c** (20.0 g, 164 mmol) in CH_2Cl_2 (328 mL) was cooled to 0 °C. After the addition of pyridine (19.9 mL, 246 mmol) and 3-chloropropanoyl chloride (20.4 mL, 213 mmol) at 0 °C, the mixture was stirred at room temperature for 2 h. A saturated solution of NH_4Cl was added and the aqueous layer was extracted with CH_2Cl_2. The organic layer was washed with a saturated solution of NaCl, dried over Na_2SO_4 and evaporated. The residue was purified by column chromatography (hexane/EtOAc) to afford 22.0 g of 3,4-dimethylphenyl 3-chloropropanoate (63% yield).

A mixture of 3,4-dimethylphenyl 3-chloropropanoate (22.0 g, 103 mmol) and aluminum chloride (55.2 g, 414 mmol) was heated at 180 °C for 2 h. After cooling, ice, concentrated HCl and CH_2Cl_2 were added and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were washed with H_2O and a saturated solution of NaCl, dried over Na_2SO_4 and evaporated. The remaining solid was collected by filtration and washed with EtOAc to afford 14.1 g of 7-hydroxy-4,5-dimethylindan-1-one (78% yield).

A mixture of 7-hydroxy-4,5-dimethylindan-1-one (5.00 g, 28.4 mmol), K_2CO_3 (5.89 g, 42.6 mmol) and diethoxyphosphorylmethyl tosylate (11.0 g, 34.1 mmol) in DMF (95 mL) was stirred at 80 °C for 4 h. After cooling, a saturated solution of NH_4Cl was added. DMF was evaporated and the remaining aqueous solution was extracted with EtOAc. The organic layer was washed with a saturated solution of NaCl, dried over Na_2SO_4 and evaporated. The residue was purified by column chromatography (hexane/EtOAc) to afford 7.26 g of **13c** (78% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.36 (6H, t, J = 7.1 Hz), 2.16 (3H, s), 2.33 (3H, s), 2.62–2.66 (2H, m), 2.92–2.97 (2H, m), 4.32 (4H, dq, J = 7.1, 7.1 Hz), 4.40 (2H, d, J = 9.4 Hz), 6.67 (1H, s). IR (KBr): 1705, 1244, 1029 cm^{-1}. MS (FAB): m/z 327 $(M+H)^+$.

4.2.2. Diethyl {[(6,7-dimethyl-8*H*-indeno[1,2-*d*][1,3]thiazol-4-yl)oxy]methyl}phosphonate (14c)

A mixture of **13c** (7.26 g, 22.2 mmol) and $CuBr_2$ (9.92 g, 44.4 mmol) in EtOH (74 mL) was heated at 60 °C for 2 h. After cooling, a saturated solution of $NaHCO_3$ was added. EtOH was evaporated and the remaining aqueous solution was extracted with EtOAc. The organic layer was washed with a saturated solution of NH_4Cl, H_2O and a saturated solution of NaCl, dried over Na_2SO_4 and evaporated to afford 9.00 g of diethyl {[(2-bromo-6,7-dimethyl-3-oxo-2,3-dihydro-1*H*-inden-4-yl)oxy]methyl}phosphonate (99% yield).

Formamide (2.65 mL, 66.6 mmol) was added dropwise to a suspension of P_2S_5 (2.96 g, 13.3 mmol) in THF (89 mL). The mixture was stirred at room temperature for 4 h and filtered. The filtrate was added to a solution of diethyl {[(2-bromo-6,7-dimethyl-3-oxo-2,3-dihydro-1*H*-inden-4-yl)oxy]methyl}phosphonate (9.00 g, 22.2 mmol) in THF (22 mL) and the mixture was refluxed for 2 h. After cooling, a saturated solution of $NaHCO_3$ was added. THF was evaporated and the remaining aqueous solution was extracted with CH_2Cl_2. The organic layer was washed with a saturated solution of NaCl, dried over Na_2SO_4 and evaporated. The residue was purified by column chromatography (EtOAc/MeOH). Recrystalliza-

tion from EtOAc–hexane produced 4.70 g of **14c** (58% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.35 (6H, t, J = 7.0 Hz), 2.24 (3H, s), 2.34 (3H, s), 3.74 (2H, s), 4.31 (4H, dq, J = 7.0, 7.0 Hz), 4.63 (2H, d, J = 8.6 Hz), 6.88 (1H, s), 8.79 (1H, s). IR (KBr): 2980, 1236, 1106, 1047, 1024 cm^{-1}. MS (FAB): m/z 368 (M+H)$^+$.

4.2.3. Ethyl (2*S*,6*S*)-4-{[(6,7-dimethyl-8*H*-indeno[1,2-*d*][1,3]thiazol-4-yl)oxy]methyl}-2,6-dimethyl-7-oxo-8-oxa-3,5-diaza-4-phosphadecan-1-oate 4-oxide (15c)

Bromotrimethylsilane (3.59 mL, 27.2 mmol) was added to a solution of **14c** (2.00 g, 5.44 mmol) in CH_2Cl_2 (54 mL). The mixture was stirred at room temperature for 16 h and the volatiles were evaporated.

The remaining residue was dissolved in CH_2Cl_2 (54 mL). After the addition of oxalyl chloride (2.37 mL, 27.2 mmol) and DMF (one drop), the mixture was stirred at room temperature for 1 h. The reaction mixture was concentrated and azeotroped with toluene.

The remaining residue was dissolved in CH_2Cl_2 (54 mL) and cooled to −20 °C. After the addition of ethyl L-alaninate methanesulfonate (5.80 g, 27.2 mmol) and *N*,*N*-diisopropylethylamine (9.48 mL, 54.4 mmol) at −20 °C, the mixture was allowed to warm to room temperature and stirred for 4 h. A saturated solution of $NaHCO_3$ was added and the aqueous phase was extracted with CH_2Cl_2. The organic layer was washed with a saturated solution of NaCl, dried over Na_2SO_4 and evaporated. The residue was purified by column chromatography (EtOAc/MeOH) to afford 1.51 g of **15c** (55% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.22 (3H, t, J = 7.0 Hz), 1.23 (3H, t, J = 7.0 Hz), 1.42 (3H, d, J = 7.0 Hz), 1.48 (3H, d, J = 7.0 Hz), 2.24 (3H, s), 2.32 (3H, s), 3.75 (2H, s), 4.07–4.23 (6H, m), 4.30 (1H, dd, J = 12.5, 9.6 Hz), 4.37–4.45 (1H, m), 4.38 (1H, dd, J = 12.5, 9.0 Hz), 4.95–5.07 (1H, m), 6.84 (1H, s), 8.86 (1H, s). IR (KBr): 3199, 1748, 1193, 1159 cm^{-1}. MS (FAB): m/z 510 (M+H)$^+$.

4.2.4. Ethyl (2*S*,6*S*)-2,6-dimethyl-4-{[(7-methyl-8*H*-indeno[1,2-*d*][1,3]thiazol-4-yl)oxy]methyl}-7-oxo-8-oxa-3,5-diaza-4-phosphadecan-1-oate 4-oxide (15a)

The title compound was prepared according to the procedure for **15c** from 4-methylphenol **12a** as a starting material instead of 3,4-dimethylphenol **12c**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.20–1.26 (6H, m), 1.43 (3H, d, J = 7.0 Hz), 1.48 (3H, d, J = 7.0 Hz), 2.36 (3H, s), 3.75 (2H, s), 4.06–4.26 (6H, m), 4.29–4.46 (3H, m), 4.95–5.07 (1H, m), 6.96 (1H, d, J = 8.2 Hz), 7.03 (1H, d, J = 8.2 Hz), 8.91 (1H, s). MS (FAB): m/z 496 (M+H)$^+$.

4.2.5. Ethyl (2*S*,6*S*)-4-{[(7-ethyl-8*H*-indeno[1,2-*d*][1,3]thiazol-4-yl)oxy]methyl}-2,6-dimethyl-7-oxo-8-oxa-3,5-diaza-4-phosphadecan-1-oate 4-oxide (15b)

The title compound was prepared according to the procedure for **15c** from 4-ethylphenol **12b** as a starting material instead of 3,4-dimethylphenol **12c**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.20–1.25 (6H, m), 1.28 (3H, t, J = 7.4 Hz), 1.43 (3H, d, J = 7.0 Hz), 1.48 (3H, d, J = 7.0 Hz), 2.70 (2H, q, J = 7.4 Hz), 3.79 (2H, s), 4.05–4.24 (6H, m), 4.29–4.44 (3H, m), 4.95–5.06 (1H, m), 6.97 (1H, d, J = 8.6 Hz), 7.05 (1H, d, J = 8.6 Hz), 8.88 (1H, s). MS (FAB): m/z 510 (M+H)$^+$.

4.2.6. Isopropyl (2*S*,6*S*)-4-{[(6,7-dimethyl-8*H*-indeno[1,2-*d*][1,3]thiazol-4-yl)oxy]methyl}-2,6,9-trimethyl-7-oxo-8-oxa-3,5-diaza-4-phosphadecan-1-oate 4-oxide (15d)

The title compound was prepared according to the procedure for **15c** utilizing isopropyl L-alaninate hydrochloride instead of ethyl L-alaninate methanesulfonate. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.17–1.25 (12H, m), 1.42 (3H, d, J = 6.8 Hz), 1.47 (3H, d, J = 7.3 Hz), 2.25 (3H, s), 2.33 (3H, s), 3.76 (2H, s), 4.10–4.21 (2H, m), 4.27–4.44 (3H, m), 4.96–5.09 (3H, m), 6.86 (1H, s), 8.89 (1H, s). MS (FAB): m/z 538 (M+H)$^+$.

4.2.7. Ethyl 4-{[(6,7-dimethyl-8*H*-indeno[1,2-*d*][1,3]thiazol-4-yl)oxy]methyl}-7-oxo-8-oxa-3,5-diaza-4-phosphadecan-1-oate 4-oxide (15e)

The title compound was prepared according to the procedure for **15c** utilizing ethyl glycinate hydrochloride instead of ethyl L-alaninate methanesulfonate. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.24 (6H, t, J = 7.3 Hz), 2.25 (3H, s), 2.33 (3H, s), 3.76 (2H, s), 3.81–4.00 (4H, m), 4.17 (4H, q, J = 7.3 Hz), 4.43 (2H, d, J = 9.4 Hz), 4.66–4.76 (2H, m), 6.89 (1H, s), 8.88 (1H, s). MS (FAB): m/z 482 (M+H)$^+$.

4.2.8. Diethyl {[(7-bromo-3-oxo-2,3-dihydro-1*H*-inden-4-yl)oxy]methyl}phosphonate (7)

The title compound was prepared according to the procedure for **13c** from 4-bromophenol **6** instead of 3,4-dimethylphenol **12c**. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.37 (6H, t, J = 7.3 Hz), 2.66–2.72 (2H, m), 2.99–3.05 (2H, m), 4.33 (4H, dq, J = 7.3, 7.3 Hz), 4.42 (2H, d, J = 8.8 Hz), 6.81 (1H, d, J = 8.8 Hz), 7.66 (1H, d, J = 8.8 Hz). MS (FAB): m/z 377 (M+H)$^+$.

4.2.9. Allyl 7-[(diethoxyphosphoryl)methoxy]-1-oxoindane-4-carboxylate (8)

A mixture of **7** (20.1 g, 53.3 mmol), 2-(trimethylsilyl)ethanol (75.0 mL, 523 mmol), triethylamine (25.0 mL, 180 mmol), 1,3-bis(diphenylphosphino)propane (6.60 g, 16.0 mmol) and palladium(II) acetate (3.59 g, 16.0 mmol) in DMF (80 mL) was purged with carbon monoxide gas and heated at 70 °C for 18 h. After cooling, the mixture was filtered, concentrated and diluted with EtOAc and H_2O. The organic layer was washed with H_2O and a saturated solution of NaCl, dried over Na_2SO_4 and evaporated. The residue was purified by column chromatography (hexane/EtOAc) to afford 13.0 g of 2-(trimethylsilyl)ethyl 7-[(diethoxyphosphoryl)methoxy]-1-oxoindane-4-carboxylate (55% yield).

To a solution of 2-(trimethylsilyl)ethyl 7-[(diethoxyphosphoryl)methoxy]-1-oxoindane-4-carboxylate (13.0 g, 29.4 mmol) in THF (250 mL) was added a 1.0 M TBAF solution in THF (44.0 mL, 44.0 mmol). After stirring at room temperature for 15 h, triethylamine (12.2 mL, 87.5 mmol) and allyl bromide (7.60 mL, 87.8 mmol) were added. The mixture was stirred at room temperature for 4 h, concentrated and diluted with EtOAc and H_2O. The organic layer was washed with H_2O and a saturated solution of NaCl, dried over Na_2SO_4 and evaporated. The residue was purified by column chromatography (hexane/EtOAc) to afford 10.5 g of **8** (93% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.38 (6H, t, J = 7.0 Hz), 2.64–2.70 (2H, m), 3.43–3.48 (2H, m), 4.34 (2H, q, J = 7.0 Hz), 4.36 (2H, q, J = 7.0 Hz), 4.48 (2H, d, J = 9.0 Hz), 4.83 (2H, dt, J = 5.5, 1.2 Hz), 5.31 (1H, dd, J = 10.6, 1.2 Hz), 5.41 (1H, dd, J = 17.2, 1.2 Hz), 5.99–6.11 (1H, m), 6.93 (1H, d, J = 8.6 Hz), 8.28 (1H, d, J = 8.6 Hz). MS (FAB): m/z 383 (M+H)$^+$.

4.2.10. Allyl 4-[(diethoxyphosphoryl)methoxy]-8*H*-indeno[1,2-*d*][1,3]thiazole-7-carboxylate (9)

The title compound was prepared according to the procedure for **14c** from **8** instead of **13c**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.35 (6H, t, J = 6.8 Hz), 4.25 (2H, s), 4.33 (2H, q, J = 6.8 Hz), 4.35 (2H, q, J = 6.8 Hz), 4.67 (2H, d, J = 8.8 Hz), 4.86 (2H, dt, J = 5.9, 1.5 Hz), 5.32 (1H, dd, J = 10.3, 1.5 Hz), 5.44 (1H, ddd, J = 17.1, 2.9, 1.2 Hz), 6.03–6.13 (1H, m), 7.11 (1H, d, J = 8.8 Hz), 8.01 (1H, d, J = 8.8 Hz), 8.85 (1H, s). MS (FAB): m/z 424 (M+H)$^+$

4.2.11. Allyl 4-[(bis{[(1*S*)-2-ethoxy-1-methyl-2-oxoethyl]amino}phosphoryl)methoxy]-8*H*-indeno[1,2-*d*][1,3]thiazole-7-carboxylate (10)

The title compound was prepared according to the procedure for **15c** from **9** instead of **14c**. ^{1}H NMR (400 MHz,, $CDCl_3$): δ 1.20 (3H, t, J = 7.0 Hz), 1.21 (3H, t, J = 7.0 Hz), 1.43 (3H, d, J = 7.4 Hz), 1.47 (3H, d, J = 7.4 Hz), 4.02–4.22 (7H, m), 4.24 (2H, s), 4.42 (1H,

dd, J = 12.1, 9.8 Hz), 4.51 (1H, dd, J = 12.1, 9.4 Hz), 4.82–4.95 (3H, m), 5.29–5.34 (1H, m), 5.39–5.46 (1H, m), 6.00–6.12 (1H, m), 7.03 (1H, d, J = 8.6 Hz), 7.98 (1H, d, J = 8.6 Hz), 8.85 (1H, s). MS (FAB): m/z 566 $(M+H)^+$.

4.2.12. Ethyl (2*S*,6*S*)-4-{[(7-carbamoyl-8*H*-indeno[1,2-*d*][1,3]thiazol-4-yl)oxy]methyl}-2,6-dimethyl-7-oxo-8-oxa-3,5-diaza-4-phosphadecan-1-oate 4-oxide (11a)

A mixture of **10** (7.76 g, 13.7 mmol), tetrakis(triphenylphosphine)palladium(0) (793 mg, 0.686 mmol), triphenylphosphine (720 mg, 2.74 mmol) and pyrrolidine (2.29 mL, 27.4 mmol) in acetonitrile (150 mL) was heated at 50 °C for 2 h. The mixture was concentrated and diluted with EtOAc and a solution of $NaHCO_3$. The aqueous layer was washed with EtOAc. After the addition of 1 N hydrochloric acid, the aqueous layer was extracted with EtOAc and the organic layer was washed with saturated solution of NaCl, dried over Na_2SO_4 and evaporated. The residue was purified by column chromatography (EtOAc/MeOH) to afford 6.62 g of 4-[(bis{[(1*S*)-2-ethoxy-1-methyl-2-oxoethyl]amino}phosphoryl)methoxy]-8*H*-indeno[1,2-*d*][1,3]thiazole-7-carboxylic acid (92% yield).

A mixture of 4-[(bis{[(1*S*)-2-ethoxy-1-methyl-2-oxoethyl]amino }phosphoryl)methoxy]-8*H*-indeno[1,2-*d*][1,3]thiazole-7-carboxylic acid (1.65 g, 3.14 mmol), ammonium chloride (840 mg, 15.7 mmol), diisopropylethylamine (2.73 mL, 15.7 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (1.20 g, 6.28 mmol) and 1-hydroxybenzotriazole (849 mg, 6.28 mmol) in DMF (25 mL) was stirred at room temperature for 4 h. The mixture was concentrated and the remaining solid was collected by filtration and washed with H_2O and acetone. Recrystallization from EtOH produced 926 mg of **11a** (56% yield). ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.07–1.13 (6H, m), 1.29 (3H, d, J = 7.3 Hz), 1.30 (3H, d, J = 7.3 Hz), 3.90–4.16 (6H, m), 4.22 (2H, s), 4.32 (1H, dd, J = 12.2, 8.8 Hz), 4.40 (1H, dd, J = 12.2, 8.8 Hz), 4.94 (1H, t, J = 11.2 Hz), 5.04 (1H, t, J = 11.2 Hz), 7.17 (1H, d, J = 8.8 Hz), 7.27–7.34 (1H, m), 7.69 (1H, d, J = 8.8 Hz), 7.82–7.88 (1H, m), 9.14 (1H, s). MS (FAB): m/z 525 $(M+H)^+$.

4.2.13. Ethyl (2*S*,6*S*)-2,6-dimethyl-4-({[7-(methylcarbamoyl)-8*H*-indeno[1,2-*d*][1,3]thiazol-4-yl]oxy}methyl)-7-oxo-8-oxa-3,5-diaza-4-phosphadecan-1-oate 4-oxide (11b)

The title compound was prepared according to the procedure for **11a** utilizing methylamine hydrochloride instead of ammonium chloride. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.14–1.26 (6H, m), 1.43 (3H, d, J = 7.0 Hz), 1.47 (3H, d, J = 7.0 Hz), 3.03 (3H, d, J = 5.1 Hz), 4.03–4.24 (7H, m), 4.25 (2H, s), 4.36 (1H, dd, J = 12.1, 9.4 Hz), 4.45 (1H, dd, J = 12.1, 9.4 Hz), 4.87–4.98 (1H, m), 6.22–6.31 (1H, m), 7.00 (1H, d, J = 8.6 Hz), 7.42 (1H, d, J = 8.6 Hz), 8.88 (1H, s). MS (FAB): m/z 539 $(M+H)^+$.

4.2.14. Ethyl (2*S*,6*S*)-4-[({7-[(2,2-dimethylpropyl)carbamoyl]-8*H*-indeno[1,2-*d*][1,3]thiazol-4-yl}oxy)methyl]-2,6-dimethyl-7-oxo-8-oxa-3,5-diaza-4-phosphadecan-1-oate 4-oxide (11c)

The title compound was prepared according to the procedure for **11a** utilizing neopentylamine instead of ammonium chloride. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.01 (9H, s), 1.18–1.24 (6H, m), 1.43 (3H, d, J = 6.3 Hz), 1.47 (3H, d, J = 7.0 Hz), 3.29 (2H, d, J = 6.7 Hz), 4.03–4.22 (7H, m), 4.23 (2H, s), 4.39 (1H, dd, J = 12.1, 9.4 Hz), 4.47 (1H, dd, J = 12.1, 9.4 Hz), 4.85–5.05 (1H, m), 6.05–6.13 (1H, m), 7.02 (1H, d, J = 8.6 Hz), 7.40 (1H, d, J = 8.6 Hz), 8.86 (1H, s). MS (FAB): m/z 595 $(M+H)^+$.

References and notes

1. Wild, S.; Roglic, G.; Green, A.; Sicree, R.; King, H. *Diabetes Care* **2004**, *27*, 1047.
2. (a) Magnusson, I.; Rothman, D.; Katz, L.; Shulman, R.; Shulman, G. *J. Clin. Invest.* **1992**, *90*, 1323; (b) Consoli, A.; Nurjhan, N.; Capani, F.; Gerich, J. *Diabetes* **1989**, *38*, 550; (c) Wajngot, A.; Chandramouli, V.; Schumann, W. C.; Ekberg, K.; Jones, P. K.; Efendic, S.; Landau, B. R. *Metabolism* **2001**, *50*, 47.
3. (a) Benkovic, S. J.; deMaine, M. M. *Adv. Enzymol. Relat. Areas Mol. Biol.* **1982**, *53*, 45; (b) Gidh-Jain, M.; Zhang, Y.; van Poelje, P. D.; Liang, J.-Y.; Huang, S.; Kim, J.; Elliott, J. T.; Erion, M. D.; Pilkis, S. J.; El-Maghrabi, M. R.; Lipscomb, W. N. *J. Biol. Chem.* **1994**, *269*, 27732; (c) Zhang, Y.; Liang, J.; Huang, S.; Lipscomb, W. N. *J. Mol. Biol.* **1994**, *244*, 609; (d) Iancu, C. V.; Mukund, S.; Fromm, H. J.; Honzatko, R. B. *J. Biol. Chem.* **2005**, *280*, 19737; (e) Pilkis, S. J.; El-Maghrabi, M. R.; Pilkis, J.; Claus, T. H. *J. Biol. Chem.* **1981**, *256*, 3619.
4. (a) Wright, S. W.; Hageman, D. L.; McClure, L. D.; Carlo, A. A.; Treadway, J. L.; Mathiowetz, A. M.; Withka, J. M.; Bauer, P. H. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 17; (b) Wright, S. W.; Carlo, A. A.; Carty, M. D.; Danley, D. E.; Hageman, D. L.; Karam, G. A.; Levy, C. B.; Mansour, M. N.; Mathiowetz, A. M.; McClure, L. D.; Nestor, N. B.; McPherson, R. K.; Pandit, J.; Pustilnik, L. R.; Schulte, G. K.; Soeller, W. C.; Treadway, J. L.; Wang, I.-K.; Bauer, P. H. *J. Med. Chem.* **2002**, *45*, 3865.
5. Wright, S. W.; Carlo, A. A.; Danley, D. E.; Hageman, D. L.; Karam, G. A.; Mansour, M. N.; McClure, L. D.; Pandit, J.; Schulte, G. K.; Treadway, J. L.; Wang, I.-K.; Bauer, P. H. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2055.
6. Choe, J.-Y.; Nelson, S. W.; Arienti, K. L.; Axe, F. U.; Collins, T. L.; Jones, T. K.; Kimmich, R. D. A.; Newman, M. J.; Norvell, K.; Ripka, W. C.; Romano, S. J.; Short, K. M.; Slee, D. H.; Fromm, H. J.; Honzatko, R. B. *J. Biol. Chem.* **2003**, *278*, 51176.
7. (a) von Geldern, T. W.; Lai, C.; Gum, R. J.; Daly, M.; Sun, C.; Fry, E. H.; Abad-Zapatero, C. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1811; (b) Lai, C.; Gum, R. J.; Daly, M.; Fry, E. H.; Hutchins, C.; Abad-Zapatero, C.; von Geldern, T. W. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1807.
8. Hebeisen, P.; Kuhn, B.; Kohler, P.; Gubler, M.; Huber, W.; Kitas, E.; Schott, B.; Benz, J.; Joseph, C.; Ruf, A. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4708.
9. Kitas, E.; Mohr, P.; Kuhn, B.; Hebeisen, P.; Wessel, H. P.; Haap, W.; Ruf, A.; Benz, J.; Joseph, C.; Huber, W.; Sanchez, R. A.; Paehler, A.; Benardeau, A.; Gubler, M.; Schott, B.; Tozzo, E. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 594.
10. (a) Erion, M. D.; van Poelje, P. D.; Dang, Q.; Kasibhatla, S. R.; Potter, S. C.; Reddy, M. R.; Reddy, K. R.; Jiang, T.; Lipscomb, W. N. *Proc. Natl. Acad. Sci.* **2005**, *102*, 7970; (b) Erion, M. D.; Dang, Q.; Reddy, M. R.; Kasibhatla, S. R.; Huang, J.; Lipscomb, W. N.; van Poelje, P. D. *J. Am. Chem. Soc.* **2007**, *129*, 15480; (c) Dang, Q.; Rao Kasibhatla, S.; Reddy, K. R.; Jiang, T.; Reddy, M. R.; Potter, S. C.; Fujitaki, J. M.; van Poelje, P. D.; Huang, J.; Lipscomb, W. N.; Erion, M. D. *J. Am. Chem. Soc.* **2007**, *129*, 15491; (d) van Poelje, P. D.; Dang, Q.; Erion, M. D. *Drug Discovery Today Ther. Strateg.* **2007**, *4*, 103; (e) Yoshida, T.; Okuno, A.; Izumi, M.; Takahashi, K.; Hagisawa, Y.; Ohsumi, J.; Fujiwara, T. *Eur. J. Pharmacol.* **2008**, *601*, 192.
11. Tsukada, T.; Takahashi, M.; Takemoto, T.; Kanno, O.; Yamane, T.; Kawamura, S.; Nishi, T. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5909.
12. Tsukada, T.; Takahashi, M.; Takemoto, T.; Kanno, O.; Yamane, T.; Kawamura, S.; Nishi, T. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1004.
13. Hecker, S. J.; Erion, M. D. *J. Med. Chem.* **2008**, *51*, 2328.
14. Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. *Adv. Drug Delivery Rev.* **1997**, *23*, 3.
15. (a) Palm, K.; Stenberg, P.; Luthman, K.; Artursson, P. *Pharm. Res.* **1997**, *14*, 568; (b) Stenberg, P.; Luthman, K.; Artursson, P. *Pharm. Res.* **1999**, *16*, 205.