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Fructose-1,6-bisphosphatase Inhibitors. 2. Design, Synthesis, and Structure−Activity Relationship of a Series of Phosphonic Acid Containing Benzimidazoles that Function as 5′-Adenosinemonophosphate (AMP) Mimics

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Efforts to enhance the inhibitory potency of the initial purine series of fructose-1,6-bisphosphatase (FBPase) inhibitors led to the discovery of a series of benzimidazole analogues with human FBPase IC_{50}s < 100 nM. Inhibitor **4.4** emerged as a lead compound based on its potent inhibition of human liver FBPase (IC_{50} = 55 nM) and significant glucose lowering in normal fasted rats. Intravenous administration of **4.4** to Zucker diabetic fatty rats led to rapid and robust glucose lowering, thereby providing the first evidence that FBPase inhibitors could improve glycemia in animal models of type 2 diabetes.

Introduction

Fructose-1,6-bisphosphatase (FBPase[a]), a rate-limiting enzyme of the gluconeogenesis (GNG) pathway, has long been recognized as a potential therapeutic target for the treatment of type 2 diabetes mellitus (T2DM).[1−3] Efforts over the past two decades have produced several inhibitor series,[4−10] but no reports prior to our work of glucose lowering activity in animal models of diabetes. Using a structure-guided drug design strategy,[3] we discovered CS-917 (**2a**, Figure 1), a diamide prodrug[11] of the potent and specific FBPase inhibitor MB05032 (**2**),[12] which demonstrated robust glucose lowering activity in animal models[13,14] and in patients with T2DM.[15] This inhibitor series arose from our initial discovery of a series of 8,9-disubstituted purines that functioned as AMP mimetics capable of retaining the key binding interactions of AMP in the allosteric binding site of FBPase while also forming additional interactions designed to enhance potency and specificity[16] (Figure 2). An important step in the transformation of this modestly potent purine series (**1a**, IC_{50} = 0.8 μM) into the thiazole series of compounds (**2**, IC_{50} = 0.016 μM) was our discovery of a series of benzimidazole-based inhibitors. Herein we report the design, synthesis, and structure−activity relationships (SAR) of this compound series and results from a study in the Zucker diabetic fatty rat, demonstrating that FBPase inhibitors produce rapid and significant glucose lowering in an animal of severe type 2 diabetes.

Inhibitor Design. In an effort to enhance the potency and possibly the specificity of the purine series of FBPase inhibitors, we focused our attention on the N^1 and N^3 atoms of the purine ring. Crystallographic data suggests that neither N^1 nor N^3 appeared to form a hydrogen bond with the FBPase binding site (Figure 2). Consequently, removal of one or both of the nitrogens was considered unlikely to result in a loss of binding affinity and more likely to improve inhibitor potency by minimizing any losses due to desolvation. Moreover, because many purine-containing nucleotides form hydrogen bonds to one or both nitrogens, their removal could enhance specificity for the AMP binding site of FBPase over other AMP binding sites. The resulting benzimidazoles were further optimized by attaching lipophilic groups to the ring in order to produce favorable van der Waals interactions with residues such as ^{177}Met and ^{160}Val.

Synthesis of Benzimidazole Analogues. The N^1-substituted 4-aminobenzimidazole phosphonate analogues were prepared from 5-diethylphosphono-2-furaldehyde (**3**)[3] via a four-step procedure as shown in Scheme 1. The cyclization of aldehyde **3** with 3-nitro-benzene-1,2-diamine using the $FeCl_3$-promoted cyclization reaction developed for purine synthesis[17] gave benzimidazole **4** in 81% yield. The R^1 group was introduced via either Mitsunobu reactions with alcohols or alkylation reactions with alkyl bromides using sodium hydride as the base. The nitro group was subsequently reduced to its corresponding amino group under hydrogenation conditions to give compounds **5**, and the phosphonate diethyl esters were cleaved using TMSBr to yield compounds **1.1**−**1.17**.

The C5-halo benzimidazole analogues were readily prepared from compound **5a** (wherein R^1 is isobutyl group) through direct halogenation reactions (Scheme 2). Thus, treatment of **5a** with NBS gave both C5-bromo (**6a**) and C7-bromo (**7a**) isomers in 21 and 25% yields, respectively. Similarly, chlorination of compound **5a** with NCS produced compounds **6b** and **7b** in 49 and 31% yields, respectively.

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[a] Abbreviations: FBPase, fructose 1,6-bisphosphatase; OBAV, oral bioavailability; T2DM, type 2 diabetes mellitus; EGP, endogenous glucose production; GNG, gluconeogenesis; AMP, 5′-adenosinemonophosphate; ZDF rats, Zucker diabetic fatty rats.

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Treatment of compounds **6a**−**b** with TMSBr gave compounds **2.7** and **2.5**, respectively. Compound **6a** was also used to prepare other C5 analogues, as depicted in Scheme 3. Stille coupling of **6a** with either vinyltin or allyltin gave compounds **8a** and **8b**, respectively. Hydrogenation of compounds **8a**−**b** followed by TMSBr-mediated ester deprotection gave compounds **2.1** and **2.2**, respectively.

The TMSBr-mediated ester deprotection of compounds **7a** and **7b** gave compounds **2.8** and **2.6**, respectively (Scheme 4).

The four-step sequence described in Scheme 1 was also used to prepare other benzimidazole analogues, as exemplified in Scheme 5. Cyclization of **9a**−**b** with aldehyde **3** under the $FeCl_3$−SiO_2 reaction conditions gave compounds **10a** and **10b**, respectively. N^1-alkylations of compounds **10a**−**b** via Mitsunobu reactions followed by hydrogenation reactions gave compounds **11a** and **11b**, respectively, and TMSBr-mediated removal of the phosphonate esters gave compounds **2.3** and **2.9**, respectively.

Treatment of **11a** with boron tribromide resulted in removals of both phosphonate diethyl esters and the methyl group from the C5-methoxy leading to compound **2.4** (Scheme 6). On the other hand, N^1-alkylation of compound **10b** with various alkyl bromides, followed by hydrogenation and TMSBr-mediated deprotection of phosphonate esters gave compounds **2.10**, **2.11**, and **2.12** (Scheme 7).

Double halogenation of compound **5a** with excess NBS or NCS followed by TMSBr-mediated phosphonate ester deprotection gave the 5,7-dihalo-4-aminobenzimidazole analogues **3.1** and **3.2**, respectively (Scheme 8).

Halogenation of **11b** with either NBS or NCS followed by TMSBr-mediated removal of phosphonate esters gave compounds **3.3** and **3.4**, respectively (Scheme 9).

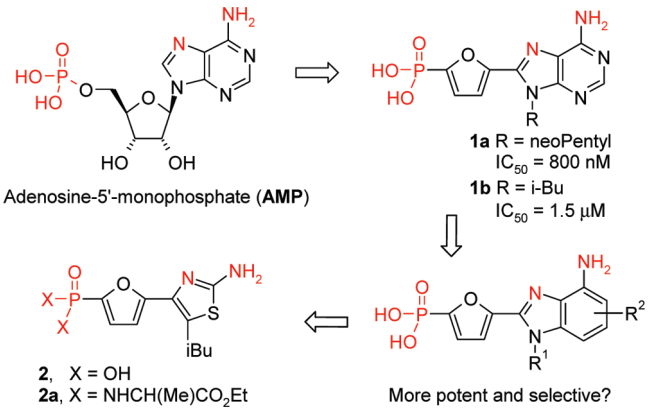
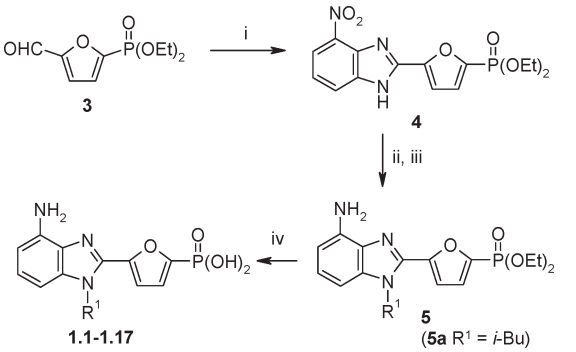


Figure 1. AMP Mimics as FBPase inhibitors.

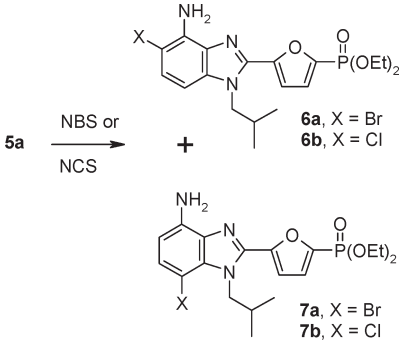
Other 1,2-phenylenediamines (**13a**−**b**) were also subjected to the four-step sequence described in Scheme 1 to give fully

Scheme 1[a]

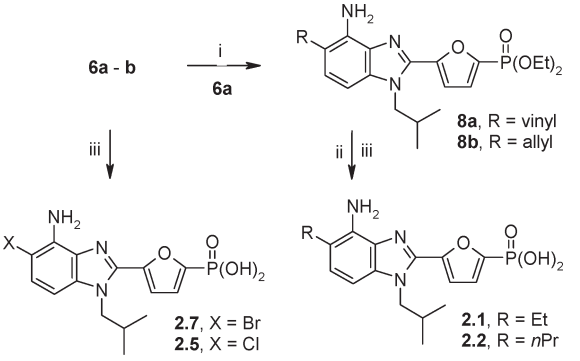


[a] Conditions and reagents: (i) 3-nitro-benzene-1,2-diamine, $FeCl_3$−SiO_2, DMSO, 80 °C, 2 h; (ii) (a) R^1-OH, PPh_3, DEAD or (b) R^1Br, NaH, DMF; (iii) H_2, Pd−C, EtOH; (iv) TMSBr, CH_2Cl_2.

Scheme 2



Scheme 3[a]



[a] Conditions and reagents: (i) $Pd(PPh_3)_4$, DMF vinyl- or allyl-$SnBu_3$; (ii) H_2, Pd−C, EtOH; (iii) TMSBr, CH_2Cl_2.

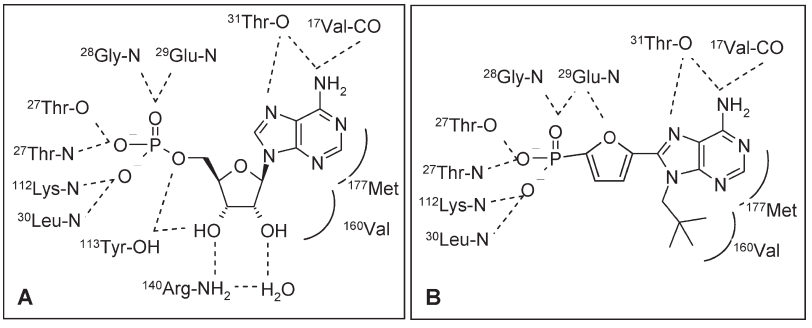
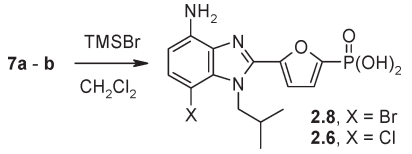
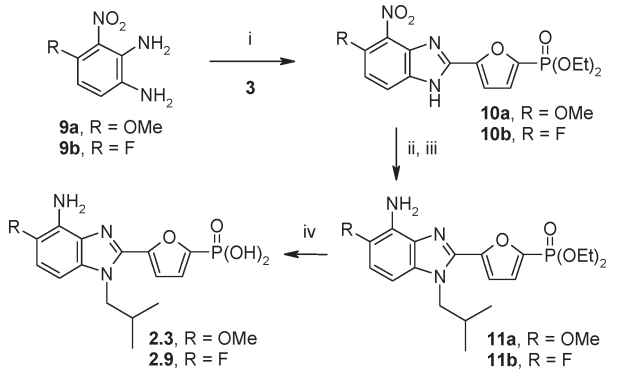


Figure 2. Purine **1a** (B) mimicking AMP (A) interactions with human FBPase.

Scheme 4

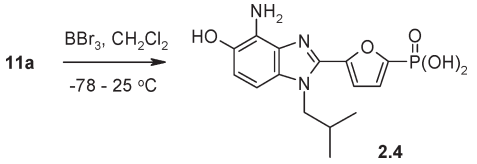


Scheme 5[a]

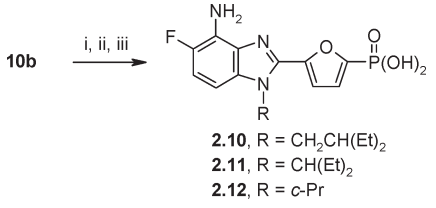


[a] Conditions and reagents: (i) $FeCl_3-SiO_2$, DMSO, 80 °C; (ii) *i*BuOH, PPh_3, DEAD; (iii) H_2, Pd−C, EtOH; (iv) TMSBr, CH_2Cl_2.

Scheme 6

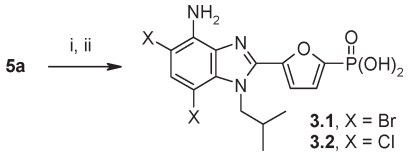


Scheme 7[a]



[a] Conditions and reagents: (i) NaH, DMF, RBr; (ii) H_2, Pd−C, EtOH; (iii) TMSBr, CH_2Cl_2.

Scheme 8[a]

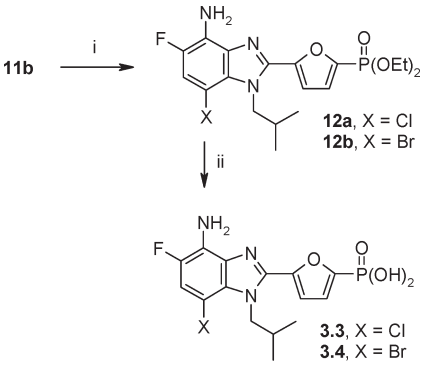


[a] Conditions and reagents: (i) NBS or NCS, DMF; (ii) TMSBr, CH_2Cl_2.

substituted benzimidazole analogues **3.5** and **3.6** as shown in Scheme 10.

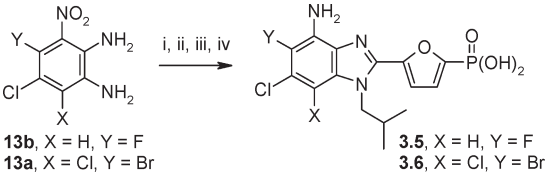
To fine-tune the 4-amino-5-fluorobenzimidazole series, the C7 SAR was rapidly explored using compound **12b** as depicted in Schemes 11, 12, and 13. Stille coupling of **12b** with vinyltin gave compound **14**. TMSBr-mediated removal of phosphonate ester gave analogue **3.8**. Alternatively, **14**

Scheme 9[a]



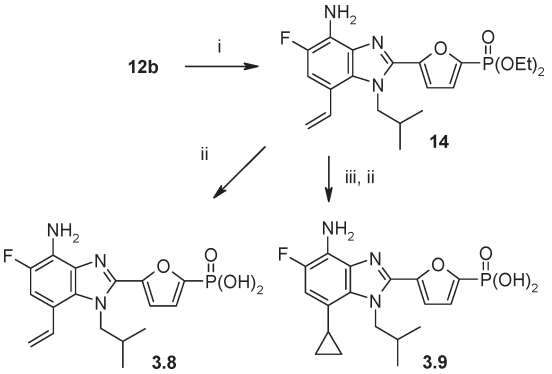
[a] Conditions and reagents: (i) NBS or NCS, DMF; (ii) TMSBr, CH_2Cl_2.

Scheme 10[a]



[a] Conditions and reagents: (i) **3**, $FeCl_3-SiO_2$, DMSO, 80 °C; (ii) *i*BuOH, PPh_3, DEAD; (iii) H_2, Pd−C, EtOH; (iv) TMSBr, CH_2Cl_2.

Scheme 11[a]



[a] Conditions and reagents: (i) $Pd(PPh_3)_4$, DMF, vinyl-$SnBu_3$; (ii) TMSBr, CH_2Cl_2; (iii) CH_2I_2, Zn.

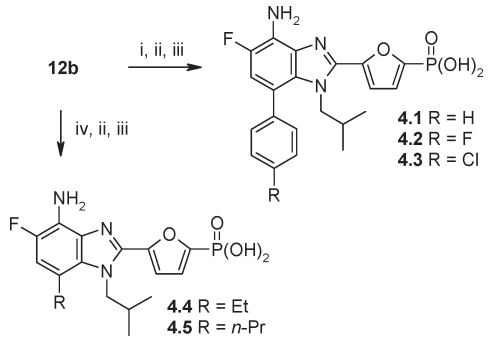
was subjected to Simmons−Smith reaction followed by TMSBr-mediated phosphonate ester deprotection yielding analogue **3.9**.

Suzuki couplings of **12b** with various arylboronic acids followed by hydrogenation and TMSBr-mediated deprotection of phosphonate esters gave analogues **4.1**, **4.2**, and **4.3** (Scheme 12). Alternatively, Stille couplings of **12b** with either vinyltin or allyltin followed by hydrogenation and TMSBr-mediated deprotection of phosphate esters gave analogues **4.4** and **4.5**.

Sonogashira couplings of **12b** with various alkynes followed by hydrogenation and TMSBr-mediated deprotection of phosphonate esters gave analogues **4.6**−**4.9**, Scheme 13.

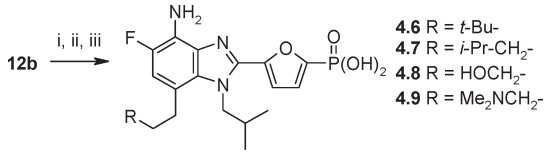
Two methods were used to prepare prodrugs of lead FBPase inhibitor **4.4** as depicted in Schemes 14 and 15. The bisPOM[18] and bisPOC[19] phosphonic esters of **4.4** were obtained via direct alkylation with either POM-I or POC-I to produce prodrugs **5.1** and **5.3**, respectively. The SATE[20] and other phosphonic esters[21] were prepared using the

Scheme 12[a]



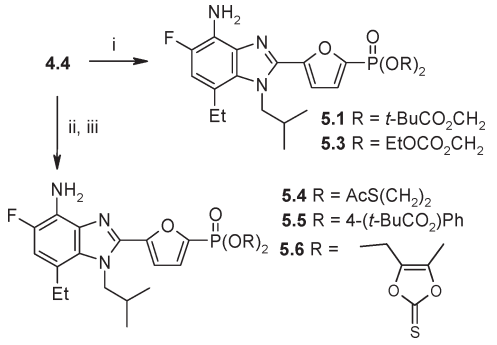
[a] Conditions and reagents: (i) $Pd(PPh_3)_4$, DMF, *p*-R-C_6H_4−$B(OH)_2$; (ii) H_2, Pd−C, EtOH; (iii) TMSBr, CH_2Cl_2; (iv) $Pd(PPh_3)_4$, DMF vinyl- or allyl-$SnBu_3$.

Scheme 13[a]



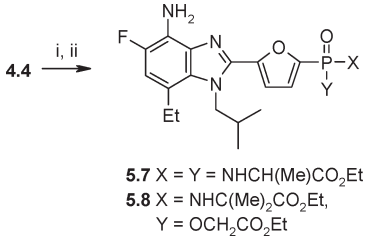
[a] Conditions and reagents: (i) $Pd(PPh_3)_4$, DMF, R-C≡CH; (ii) H_2, Pd−C, EtOH; (iii) TMSBr, CH_2Cl_2.

Scheme 14[a]



[a] Conditions and reagents: (i) R-I, Hunig's base, DMF; (ii) SO_2Cl_2; (iii) ROH, Hunig's base, DMF.

Scheme 15[a]



[a] Conditions and reagents: (i) SO_2Cl_2; (ii) L-alanine ethyl ester (3 equiv) or ethyl glycolate (0.9 equiv) followed by 2-methylalanine ethyl ester (2 equiv).

phosphonic dichloride coupling method. Treatment of **4.4** with thionyl chloride generated its corresponding phosphonic dichloride, which was treated with $AcSCH_2CH_2OH$ or 4-(*t*-$BuCO_2$)Ph−CH_2OH or 4-hydroxymethyl-5-methyl-[1,3]-dioxole-2-thione to give prodrugs **5.4**, **5.5** and **5.6**, respectively.

The phosphonic dichloride method was also used to prepare the amino acid containing prodrugs **5.7** −**5.8** as shown in Scheme 15. Coupling of the phosphonic dichloride of **4.4** with L-alanine ethyl ester in the presence of Hunig's base gave the diamide prodrug **5.7**, while stepwise coupling of the phosphonic dichloride of **4.4** first with 2-methylalanine ethyl ester and then with ethyl glycolate gave prodrug **5.8**.

In Vitro Evaluations of Benzimidazole Phosphonate Analogues. Replacing the N^1 and N^3 nitrogen atoms in compound **1b** with CH generates the benzimidazole analogue **1.4**, which exhibited identical FBPase inhibitory potency as **1b**. This observation validated our hypothesis that neither N^1 nor N^3 of our initial purine series of FBPase inhibitors are required for binding affinity. To explore the SAR of the benzimidazole scaffold, we next prepared analogues wherein we attached various alkyl and aryl groups to the benzimidazole base. The analogues were evaluated as FBPase inhibitors of both human liver and rat liver FBPase. In addition, inhibitors were tested in primary rat hepatocytes for their ability to inhibit glucose production (Table 1). Numerous N^1-benzimidazole analogues were tested, but only **1.5** and **1.6** showed slight improvement in potency compared to **1.4**. The activity of analogues **1.1**−**1.17** indicates that medium size alkyl groups such as cyclopropylmethyl and cyclobutylmethyl are preferred, while small (e.g., methyl) or large lipophilic groups (benzyl, 4-*tert*-butyl-benzyl) are not well tolerated. Consistent with previous reports, rat FBPase inhibitory potency was 2- to 25-fold less than human FBPase potency. In general, the rat liver FBPase potency for these compounds correlates well with the relative inhibitory potency measured in the primary rat hepatocyte assay, e.g., compounds **1.4** and **1.10** are potent inhibitors of rat liver FBPase and both of them showed good efficacy at inhibiting glucose production in rat hepatocytes. On the other hand, compounds **1.3**, **1.6**, and **1.7** are equipotent compared to compound **1.10** against rat liver FBPase but are 2-fold less potent in the cellular assay. It is likely that these phosphonic acids penetrate cells via organic anion transporters (OATs).[22] Therefore, both rat liver FBPase IC_{50} and OAT substrate specificity may be determinants of potency at the cellular level.

Compared to a purine, the benzimidazole scaffold presents two additional positions for optimization. To improve potency of **1.4**, C5 and C7 SAR was explored with various substituents and results are summarized in Table 2.

The C5 SAR was explored with both electron-donating (e.g., alkyl, hydroxy and alkoxy) and electron-withdrawing (e.g., Cl) groups, analogues **2.1**−**2.5**, **2.7**, and **2.9**, Table 2. Addition of alkyl groups at C5 led to slight decrease in potency, while C5-OMe and C5-OH analogues gave modest improvement in potency. Halogenation at C5-position produced the most significant increase in potency, with fluoro being the best, followed by chloro and bromo groups. On the other hand, halogenation at the C7-position (analogues **2.6** and **2.8**) was less effective compared to C5-halogenation. Having identified C5−F as a preferred group, several N^1-substituents were then explored in order to improve FBPase inhibitory potency further. Increasing lipophilicity via introduction of additional methyl groups to the N^1-isobutyl group in compound **2.9** did not enhance potency (compounds **2.10** and **2.11**, Table 2). On the other hand, converting the two methyl groups into a cyclopropyl group in compound **2.9** produced 2-fold improvement in potency (compound **2.12**, Table 2). To optimize the benzimidazole scaffold further, combination of subsituents at C5-, C6-, and C7-positions were explored and results are summarized in Table 3 and 4.

Table 1. SAR of N^1-Benzimidazole Analogues[a]

	R	HL IC_{50}, μM	RL IC_{50}, μM	EC_{50}, μM
1b		1.5		
1.1	Me	6	20	100
1.2	Et	2.25	40	110
1.3	nPr	1.1	2	37
1.4	*i*Bu	1.5	4	20
1.5	cyclopropyl-CH_2−	0.8	20	100
1.6	cyclobutyl-CH_2−	0.8	2	40
1.7	cyclopentyl-CH_2−	1.5	2	45
1.8	cyclohexyl-CH_2−	2.5	20	100
1.9	cycloheptyl-CH_2−	3.25	20	100
1.10	norbonyl	1	2	25
1.11	benzyl	5	20	100
1.12	4-*t*Bu-benzyl	9.5	20	100
1.13	4-CF_3-benzyl	7	20	100
1.14	4-Ph-benzyl	2.5	20	100
1.15	3-furnyl-CH_2−	4.2	20	100
1.16	3-HO-benzyl	1.85	20	100
1.17	3-thienyl-CH_2−	4	20	100

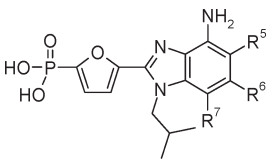
[a] HL, human liver FBPase; RL, rat liver FBPase; EC_{50}, inhibition of glucose production in primary rat hepatocytes.

Table 2. SAR of R^5- and R^7-Benzimidazole Analogues[a]

	R^1	R^5	R^7	HL IC_{50}, μM	RL IC_{50}, μM	EC_{50}, μM
1.4	*i*Bu	H	H	1.5	4	20
2.1	*i*Bu	Et	H	2.5	20	50
2.2	*i*Bu	nPr	H	3	20	50
2.3	*i*Bu	MeO	H	0.7	2	16
2.4	*i*Bu	HO	H	0.5	20	25
2.5	*i*Bu	Cl	H	0.2	2	15
2.6	*i*Bu	H	Cl	0.9	NT	24
2.7	*i*Bu	Br	H	0.4	2	14
2.8	*i*Bu	H	Br	0.4	2	14
2.9	*i*Bu	F	H	0.1	2.28	2.4
2.10	$(Et)_2CHCH_2$−	F	H	0.15	0.85	5
2.11	$(Et)_2CH$−	F	H	0.85	2.5	9.5
2.12	*c*Pr-CH_2−	F	H	0.055	2.18	3.3

[a] HL, human liver FBPase; RL, rat liver FBPase; EC_{50}, inhibition of glucose production in primary rat hepatocytes.

Table 3. SAR of R^5-, R^6-, and R^7-Benzimidazole Analogues[a]



	R^5	R^6	R^7	HL IC_{50}, μM	RL IC_{50}, μM	EC_{50}, μM
2.9	F	H	H	0.1	2.28	2.4
3.1	Br	H	Br	1	2	14
3.2	Cl	H	Cl	0.45	2	14
3.3	F	H	Cl	0.1	2	3.6
3.4	F	H	Br	0.13	2.09	5
3.5	F	Cl	H	0.225	2	16
3.6	Br	Cl	Cl	10	NT	NT
3.7	F	H	vinyl	0.28	0.9	25
3.8	F	H	cPr	0.06	0.35	1.5

[a] HL, human liver FBPase; RL, rat liver FBPase; EC_{50}, inhibition of glucose production in primary rat hepatocytes.

The C5- and C7-dibromo and -dichloro analogues **3.1** and **3.2** are much weaker compounds compared to **2.9**, while compounds **3.3** and **3.4** with either a chloro or a bromo group at the C7-position in addition to the fluoro group at C5-position have similar activity compared to **2.9**. The 5-fluoro-6-chloro analogue **3.5** is 2-fold weaker than **2.9**, but the 5-bromo-6,7-dichloro analogue **3.6** lost significant FBPase inhibition. These analogues indicate that the C5-fluoro substitution is preferred, thus two analogues were prepared to explore the C7-position in addition to the presence of a C5-fluoro group. The C7-vinyl analogue **3.7** is a 2-fold weaker FBPase inhibitor compared to compound **2.9**, while the C7-cyclopropyl **3.8** is ca. 2-fold more potent than compound **2.9**. This observation suggests further SAR exploration at C7-position is warranted.

Various R^7 analogues of compound **3.8** were prepared and evaluated, Table 4. Introduction of aryl groups did not improve potency against human liver FBPase (compounds **4.1**−**4.3**), and more importantly all three compounds failed to lower blood glucose levels after intravenous (iv) administration. Thus, alkyl groups were explored (compounds **4.4**−**4.10**). The C7-ethyl analogue **4.4** is equipotent as compound **3.8**, and it produced significant glucose-lowering effect after iv administration. On the basis of the potent glucose-lowering effects and ease of synthesis, compound **4.4** was selected for further evaluations.

Selectivity Evaluations. The selectivity of compound **4.4** against five common enzymes with an AMP-binding site were measured and compared to purine analogue **1b**, Table 5.

Benzimidazole **4.4** inhibits human liver FBPase with a 10-fold improvement compared to the most potent purine FBPase inhibitor **1b**. Moreover, no effects were observed at **4.4** concentrations 1000-fold above its FBPase IC_{50} for all five AMP-binding enzymes and no significant effects were observed in a selectivity screen against >70 enzymes, ion channels, and receptors (Panlabs, Bothel, WA) at 10 μM of **4.4**.

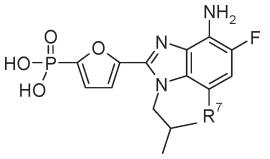
Inhibition was demonstrated to occur through binding to the AMP binding site based results from a ^{3}H-labeled AMP displacement assay, which showed that compound **4.4** dose-dependently displaced AMP.[16,23] The cellular efficacy of these benzimidazole AMP mimetics was assessed in a glucose production assay using freshly isolated primary rat hepatocytes.[1] Compound **4.4** almost completely blocked glucose production in primary rat hepatocytes at doses of 10 and 100 μM, Figure 3.

In Vivo Glucose Lowering Effects. To test whether FBPase inhibitors effectively lowered glucose in a rodent model of type 2 diabetes, we evaluated compound **4.4** in the Zucker diabetic fatty (ZDF) rat. Administering **4.4** for 6 h by tail vein infusion to 5 h-fasted ZDF rats (12 weeks of age) at a dose of 10 mg/kg/h led to significant glucose lowering (shown in Figure 4), which provided a strong piece of evidence that FBPase inhibitor would be effective in treating diabetic hyperglycemia.

Even though compound **4.4**, being a phosphonic acid, was expected to have low oral bioavailability (OBAV), it was tested for oral efficacy in the normal fasted rat assay at doses up to 100 mg/kg; plasma glucose levels were unchanged in this study, indicating that **4.4** indeed has limited oral bioavailability. The dianionic character of **4.4**, similar to other phosphonic acids, likely limits the oral absorption of compound **4.4**. Consequently, we prepared various phosphonic acid prodrugs[21] and evaluated them for OBAV or oral efficacy (Table 6).

The classic acyloxyalkyl ester prodrug **5.1** did not exhibit significant improvement in OBAV compared to **4.4**, most

Table 4. SAR of R^7-Benzimidazole Analogues of Compound **3.8**[a]



	R^7	HL IC_{50}, μM	RL IC_{50}, μM	EC_{50}, μM	G-LOW, %
3.8	cPr	0.06	0.35	1.5	64
4.1	Ph	0.09	0.9	18	0
4.2	4-F-Ph	0.18	1.3	21	0
4.3	4-Cl-Ph	0.09	2	>25	NT
4.4	Et	0.055	0.55	3.5	64
4.5	*n*Pr	0.1	0.65	5.5	60
4.6	tBu$(CH_2)_2-$	0.21	1.6	18	28
4.7	$(Me)_2CH(CH_2)_3-$	0.1	2.1	23	NT
4.8	$HO(CH_2)_3-$	0.08	0.45	1	63
4.9	$(Me)_2N(CH_2)_3-$	0.055	0.4	9	44
4.10	$Cl(CH_2)_4-$	0.07	0.5	3.1	47

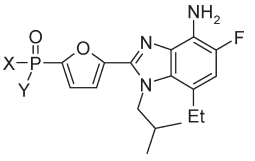
[a] HL, human liver FBPase; RL, rat liver FBPase; EC_{50}, inhibition of glucose production in primary rat hepatocytes; G-LOW, glucose lowering in the normal fasted rats after iv administration.

Table 5. Selectivity of Purine **1b** and Benzimidazole **4.4**[a]

enzymes	benzimidazole **4.4**
FBPase	IC_{50} = 0.055 μM
adenosine kinase	IC_{50} > 100 μM
adenylate kinase	IC_{50} > 100 μM
AMP deaminase	IC_{50} > 100 μM
glycogen phosphorylase	EC_{50} > 100 μM
pPhosphofructokinase	EC_{50} > 100 μM

[a] Inhibition of enzymes is reported as IC_{50}, while activation of enzymes is reported as EC_{50}.

Table 6. Evaluations of Prodrugs of Benzimidazole Analogue **4.4**[a]



	MW	LogP	TPSA	RB	HB-A	HB-D	OBAV, %
4.4	381.4	2	124.32	5	7	4	1
5.1	609.6	4.35	154.92	15	11	2	2
5.2	646	3.29	128.90	16	11	0	2[b]
5.3	585.5	2.16	173.38	17	13	2	2
5.4	637.7	3.23	203.42	11	11	2	2
5.5	585.7	3.79	187.06	15	9	2	2
5.6	761.8	7.37	154.92	17	11	2	ND[c]
5.7	579.6	3.13	160.52	15	11	4	5
5.8	580.6	3.63	157.72	15	11	3	ND[d]

[a] OBAV, oral bioavailability determined using an urinary excretion assay; LogP, TPSA (total polar surface area), RB (rotatable bond), HB-A (hydrogen bond acceptor), HB-D (hydrogen bond donor) were calculated using ADME Boxes version 3.5 (Pharma Algorithm, Toronto, Canada). [b] Compound **5.2** is the HCl salt form of **5.1**. [c] Compound **5.6** was much weaker (EC_{50} > 20 μM) in the cellular assay compared to **4.4**, therefore it was not tested for OBAV. [d] Compound **5.8** did not lower glucose significantly in the normal fasted rat model after oral administration, thus OBAV was not determined.

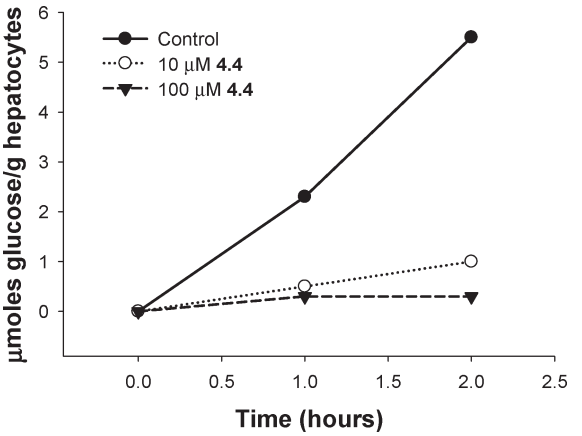


Figure 3. Inhibition of glucose production in hepatocytes by **4.4**.

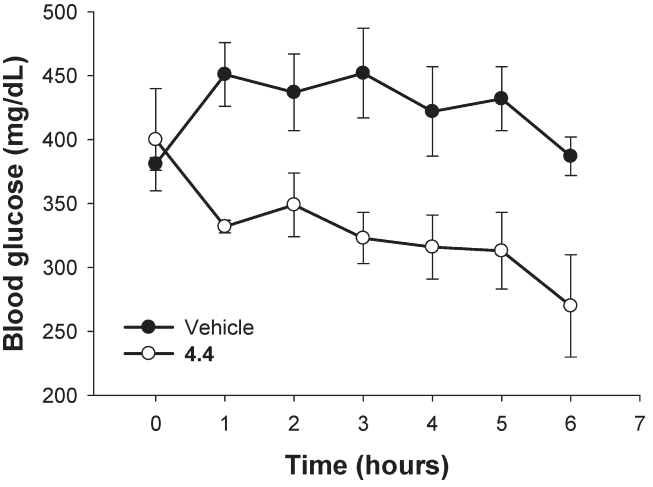


Figure 4. Glucose lowering in ZDF rats after intravenous infusion (**4.4**, 10 mg/kg/h).

likely because **5.1** is quite lipophilic and therefore likely has poor solubility. Syntheses of several other prodrugs (**5.3**−**5.8**) also did not achieve acceptable OBAV. To understand the structural features responsible for the observed low

OBAV, we determined the LogP, TPSA, RB, HB-A, and HB-D. After review of the results (Table 6), we concluded that the most likely limiting factor for the observed low OBAV was the high molecular weight (MW), given that molecules with MW > 500 tend to have lower OBAV.[24]

Conclusions

A structure-guided drug design approach was used to improve the potency and selectivity profile of an early purine phosphonate series of FBPase inhibitors. Replacements of both N^1 and N^3 atoms in the purine scaffold with carbon atoms produced new benzimidazole analogues with similar FBPase inhibitory activity relative to analogues of the purine series. Further optimization of the new benzimidazole scaffold led to the discovery of several analogues with IC_{50} values < 100 nM, which represents a significant improvement over the early purine series of FBPase inhibitors (e.g., purine **1a**, $IC_{50} = 0.8\ \mu M$). The SAR established by these benzimidazole FBPase inhibitors proved to be instrumental for the discovery of our first clinical candidate compound **2a** (a diamide prodrug of compound **2**), which will be the subject of a subsequent full paper reporting the design, synthesis, and evaluation of thiazole FBPase inhibitors. Compound **4.4** emerged as a lead inhibitor based on its potent inhibition of human liver FBPase ($IC_{50} = 55$ nM), enzyme specificity, potent cellular activity ($EC_{50} = 3.5\ \mu M$), and significant glucose-lowering (64%) in normal fasted rats. Evaluation of **4.4** in the ZDF rat demonstrated for the first time that FBPase inhibitors could significantly reduce glucose levels in an animal model of T2DM.

Experimental Section

General Methods. Glassware for moisture-sensitive reactions was flame-dried and cooled to room temperature under vacuum, and all reactions were carried out under a nitrogen atmosphere. Anhydrous solvents were purchased and used directly. TLC was performed on Merck Kieselgel 60 F_{254} plates, and flash chromatography was performed on 230−240 mesh EM Science silica gel 60. Melting points were recorded on a Thomas−Hoover capillary melting point apparatus and were uncorrected. Proton NMR spectra were obtained on a Varian Gemini-200 operating at 200 MHz and recorded in δ with tetramethylsilane (δ 0.00) as reference line internal standard. All final compounds have purities >95%, determined either by microanalyses (performed by Robertson Microlit Laboratories, Inc., Madison, NJ) or via HPLC (UV detection at 280 nm). Low resolution mass spectra were obtained from Mass Consortium Corp., San Diego, CA.

General Procedures for the Preparation of N^1-H Benzimidazoles. Diethyl [5-(4-Nitro-1*H*-benzoimidazol-2-yl)-furan-2-yl]-phosphonate (4). A solution of 3-nitro-benzene-1,2-diamine (**2**, 153 mg, 1 mmol) and diethyl (5-formyl-furan-2-yl)-phosphonate[3] (**3**, 348 mg, 1.5 mmol) in anhydrous DMSO (5 mL) was treated with $FeCl_3$−SiO_2 (20 wt %, 1.62 g, 2.0 mmol).[17] The resulting reaction mixture was heated at 80 °C for 2 h. The cooled reaction mixture was diluted with EtOAc (60 mL) and filtered through a silica gel plug (SiO_2, 5 cm × 5 cm). The silica gel was washed with EtOAc (3 × 20 mL). The combined filtrates were evaporated to dryness, and the residue was purified by flash chromatography (SiO_2, 2 cm × 10 cm, 60, 80% EtOAc−hexane, gradient elution) to give compound **4** as a yellow solid (297 mg, 81%). ^{1}H NMR ($CDCl_3$): δ 11.07 (bs, 1H), 8.24−8.14 (m, 2H), 7.47−7.26 (m, 3H) 4.33−4.22 (m, 4H), 1.43 (t, 6H, J = 7.0 Hz).

Diethyl [5-(5-Methoxy-4-nitro-1*H*-benzoimidazol-2-yl)-furan-2-yl]-phosphonate (10a). This compound was prepared in a similar manner as compound **4** from 4-methoxy-3-nitro-1,2-phenylenediamine (500 mg, 2.35 mmol) to give **10a** (502 mg, 54% yield).

Diethyl [5-(5-Fluoro-4-nitro-1*H*-benzoimidazol-2-yl)-furan-2-yl]-phosphonate (10b). This compound was prepared in a similar manner as compound **4** from 4-fluoro-3-nitro-1,2-phenylenediamine (1.0 g, 5.82 mmol) to give **10b** (1.7 g, 76% yield).

General Procedures N^1-Alkylation of Benzoimidazoles. Diethyl 5-(1-Isobutyl-4-amino-1*H*-benzoimidazol-2-yl)-furan-2-yl]-phosphonate (5a). A solution of benzimidazole **4** (365 mg, 1 mmol) in anhydrous THF (5 mL) was treated with isobutyl alcohol (1 mL, excess), triphenylphosphine (314 mg, 1.2 mmol), and DEAD (209 mg, 1.2 mmol) at room temperature for 12 h. The reaction mixture was evaporated, and the residue was purified by flash chromatography on silica gel (1 cm × 15 cm, 55% EtOAc−hexane) to give diethyl 5-(1-isobutyl-4-nitro-1*H*-benzoimidazol-2-yl)-furan-2-yl]-phosphonate (388 mg, 92%).

A solution of diethyl 5-(1-isobutyl-4-nitro-1*H*-benzoimidazol-2-yl)-furan-2-yl]-phosphonate (388 mg, 0.921 mmol) in ethanol (10 mL) was treated with palladium on carbon (10%, 40 mg) and stirred at room temperature under 1 atm of hydrogen for 24 h. The reaction mixture was filtered through a celite pad, and the filtrate was evaporated to dryness. The crude material was purified by flash chromatography on silica gel (2 cm × 15 cm, 75% EtOAc−hexane) to give **5a** as a yellow solid (372 mg, 95%).

Diethyl 5-(1-Isobutyl-4-amino-5-methoxy-1*H*-benzoimidazol-2-yl)-furan-2-yl]-phosphonate (11a). This compound was prepared in a similar manner as compound **5a** from **10a** (1.0 g, 2.5 mmol) to give **11a** (98 mg, 9.3% yield).

Diethyl 5-(1-Isobutyl-4-amino-5-fluoro-1*H*-benzoimidazol-2-yl)-furan-2-yl]-phosphonate (11b). This compound was prepared in a similar manner as compound **5a** from **10b** (1.1 g, 2.8 mmol) to give **11b** (640 mg, 56% yield). ^{1}H NMR ($CDCl_3$): δ 0.94 (d, 6H, J = 6.6 Hz), 1.37 (t, 6H, J = 8.5 Hz), 2.1−2.3 (m, 1H), 4.0−4.3 (m, 6H), 4.36 (br s, 2H), 6.65 (dd, 1H, J_1 = 8.8, J_2 = 3.7 Hz), 7.02 (dd, 1H, J_1 = 8.8, J_2 = 8.8 Hz), 7.22 (m, 1H), 7.3 (m, 1H).

General Procedures for Halogenation of Benzimidazoles. Diethyl 5-(4-Amino-5-bromo-1-isobutyl-1*H*-benzoimidazol-2-yl)-furan-2-yl]-phosphonate (6a) and Diethyl 5-(4-Amino-7-bromo-1-isobutyl-1*H*-benzoimidazol-2-yl)-furan-2-yl]-phosphonate (7a). A solution of **5a** (873 mg, 2.23 mmol) in anhydrous carbon tetrachloride (30 mL) was treated with NBS (397 mg, 2.23 mmol) and AIBN (20 mg). The resulting reaction mixture was heated to reflux for 12 h. The cooled reaction mixture was evaporated to dryness, and the residue was purified by flash chromatography on silica gel (3 cm × 15 cm, 30, 50, 75, 100% EtOAc−hexane, gradient elution) to give compounds **6a** (219 mg, 21%), **7a** (261 mg, 25%), and diethyl [5-(4-amino-5,7-dibromo-1-isobutyl-1*H*-benzoimidazol-2-yl)-furan-2-yl]-phosphonate (445 mg, 36%).

Diethyl 5-(4-Amino-5-chloro-1-isobutyl-1*H*-benzoimidazol-2-yl)-furan-2-yl]-phosphonate (6b) and Diethyl 5-(4-amino-7-chloro-1-isobutyl-1*H*-benzoimidazol-2-yl)-furan-2-yl]-phosphonate (7b). These compounds were prepared in a similar manner as compound **6a** and **7a** from **5a** (192 mg, 0.49 mmol) to give compounds **6b** (103 mg, 49% yield) and **7b** (65 mg, 31% yield).

Diethyl 5-(4-Amino-5-vinyl-1-isobutyl-1*H*-benzoimidazol-2-yl)-furan-2-yl]-phosphonate (8a). A solution of **6a** (188 mg, 0.4 mmol) in anhydrous toluene (5 mL) was treated with tributyl-vinyl-stannane (162.2 mg, 0.51 mmol) and palladium tetrakis-(triphenylphosphine) (49.6 mg, 0.04 mmol) under nitrogen. The resulting reaction mixture was heated to 80 °C for 16 h. The cooled reaction mixture was diluted with EtOAc (50 mL), washed with saturated NaF (15 mL), brine (15 mL), dried ($MgSO_4$), and evaporated. The residue was purified by flash chromatography on silica gel (2 cm × 15 cm, 75% EtOAc−hexane) to give compound **8a** (90 mg, 54%).

Diethyl 5-(4-Amino-5-allyl-1-isobutyl-1*H*-benzoimidazol-2-yl)-furan-2-yl]-phosphonate (8b). This compound was prepared in a similar manner as **8a** from **6a** (188 mg, 0.4 mmol) to give **8b** (112 mg, 65%).

Diethyl 5-(1-Isobutyl-4-amino-5-fluoro-7-chloro-1*H*-benzoimidazol-2-yl)-furan-2-yl]-phosphonate (12a). A solution of **11b** (300 mg, 0.73 mmol) in anhydrous chloroform (4 mL) was treated with NCS (147 mg, 1.1 mmol) at room temperature. After stirring for 16 h, the reaction solution was evaporated and the residue was purified by flash chromatography on silica gel (3 cm × 15 cm, 75, 85, 100% EtOAc−hexane, gradient elution) to give **12a** as a yellow solid (190 mg, 59%).

Diethyl 5-(1-Isobutyl-4-amino-5-fluoro-7-bromo-1*H*-benzoimidazol-2-yl)-furan-2-yl]-phosphonate (12b). This compound was prepared in a similar manner as compound **12a** from **11b** (150 mg, 0.37 mmol) using NBS to give **12b** (90 mg, 50% yield).

Diethyl [5-(5-Fluoro-1-isobutyl-4-nitro-7-vinyl-1*H*-benzoimidazol-2-yl)-furan-2-yl]-phosphonate (14). A solution of **12b** (80.0 g, 154 mmol) in DMF (650 mL) was treated with dichloro bis(triphenylphosphine)palladium(II) (4.5 g, 6.4 mmol). While nitrogen was bubbled through the stirred mixture, tributylvinyl tin (51.5 mL, 176 mmol) was slowly added. After the addition was complete (5 min), the mixture was warmed to 70 °C and stirred for 1 h with continued nitrogen purge. The mixture was cooled to room temperature and concentrated under reduced pressure. The resulting dark oil was dissolved in EtOAc (2.1 L) and was treated with a mixture of sodium fluoride (113 g, 2.69 mol) in water (475 mL). After stirring for 16 h, the mixture was diluted with water (350 mL) and filtered through a pad of celite in a Buchner funnel (11 cm). The layers were separated and the aqueous phase was extracted with EtOAc (800 mL). The combined organic extracts were washed with water (600 mL) and brine (600 mL) and were dried ($MgSO_4$, 125 g), filtered, and concentrated under reduced pressure to give a dark oil (88.6 g). The crude material was purified by chromatography on silica gel (1.75 kg, 6 in. diameter column), using 3:1 EtOAc−hexanes (16 L) as the eluting solvents to give **14** as a pale-orange powder (66.7 g, 92%); mp 77−78.5 °C. ^{1}H NMR (DMSO-d_6): δ 0.73 (d, 6H, J = 6.6 Hz), 1.25 (t, 3H, J = 7.0 Hz), 1.9 (m, 1H), 4.1 (m, 4H), 4.58 (d, 2H, J = 7.8 Hz), 5.7 (d, 1H, J = 9.4 Hz), 6.0 (d, 1H, J = 11.5 Hz), 7.3−7.6 (series of m, 4H).

General Procedures for the Removal of Phosphonate Diesters. Method a, TMSBr-Mediated Conditions. [5-(4-Amino-7-ethyl-5-fluoro-1-isobutyl-1*H*-benzoimidazol-2-yl)-furan-2-yl]-phosphonic Acid (4.4). A solution of **14** (57.1 g, 122 mmol) in ethanol (425 mL) was treated with palladium on carbon (4.5 g) under a continuous flow of nitrogen. The resulting mixture was stirred under one atmosphere of hydrogen for 20 h at room temperature. The reaction mixture was flushed with nitrogen, filtered through a pad of celite, and the filtrate was concentrated under reduced pressure to provide a thick orange syrup. The material was dissolved in toluene (250 mL) and filtered through a pad of celite. The filtrate was concentrated under reduced pressure to give an orange syrup (56 g, 100%). ^{1}H NMR (DMSO-d_6): δ 0.73 (d, 6H, J = 6.6 Hz), 1.05−1.35 (m, 6H), 1.85 (m, 1H), 2.82 (q, 2H, J = 7.0 Hz), 4.1 (m, 4H), 4.38 (d, 2H, J = 7.8 Hz), 5.18 (br s, 2H, exchangeable with D_2O), 6.8 (d, 1H, J = 11.5 Hz), 7.2 (m, 1H), 7.4 (m, 1H). The material was used in the next step without further purification.

A solution of the above material in dichloromethane (500 mL) was cooled to 0 °C and treated slowly with bromotrimethylsilane (112 mL, 848 mmol). The solution was allowed to warm to room temperature and was stirred for 16 h. The solvent was evaporated under reduced pressure, and the resulting residue was coevaporated with acetone (200 mL) to afford a thick orange tar. The material was suspended in water (800 mL)−acetone (150 mL), and was stirred vigorously for 2 h. The resulting solid was collected via filtration. The collected solid was washed with water (3 × 150 mL) and dried under vacuum to give crude **4.4** as a fine yellow powder (48.1 g, 103%). ^{1}H NMR (DMSO-d_6): δ 0.69 (d, 6H, J = 6.6 Hz), 1.21 (t, 3H, J = 7.2 Hz), 1.8 (m, 1H), 2.82 (q, 2H, J = 7.2 Hz), 4.38 (d, 2H, J = 7.8 Hz), 6.83 (d, 1H, J = 12.8 Hz), 7.0 (m, 1H), 7.1 (m, 1H).

The crude **4.4** (114.7 g of combined batches) was treated with a sodium hydroxide solution (1 M, 570 mL, 570 mmol) and stirred at room temperature for 2 h. The resulting cloudy, dark-orange solution was washed with EtOAc (2 × 350 mL), and the dark-orange aqueous phase was diluted with methanol (350 mL) and treated with activated carbon (Norit SA-3, 4.0 g). The mixture was filtered twice through celite and once through filter paper. The filtrate (1.4 L total volume) was treated with concentrated hydrochloric acid (48 mL, 570 mmol) dropwise with vigorous stirring. A persistent yellow precipitate formed during the second half of addition. After the addition was complete (45 min), the resulting mixture was stirred at room temperature for 2 h and then at 0 °C for 1 h. The resulting solid was collected by filtration and washed with MeOH−water (60:40, 100 mL) and water (3 × 200 mL). The solid was dried under vacuum to give **4.4** as a fine yellow−orange powder (93.4 g, 86%); mp > 220 °C. ^{1}H NMR (DMSO-d_6): δ 0.68 (d, 6H, J = 6.6 Hz), 1.21 (t, 3H, J = 7.2 Hz), 1.7−2.0 (m, 1H), 2.83 (q, 2H, J = 7.2 Hz), 4.37 (d, 2H, J = 7.4 Hz), 6.83 (d, 1H, J = 12.8 Hz), 7.02 (m, 1H), 7.12 (m, 1H). ^{13}C NMR (DMSO-d_6): δ 15.79, 19.25, 23.59, 30.47, 52.08, 112.66, 112.89, 113.1, 114.86, 115.00, 119.64, 120.10, 124.43, 124.73, 129.77, 133.49, 133.67, 143.15, 143.32, 147.65, 147.81, 148.02, 149.16, 153.66. $[MH]^+$ calcd for $C_{17}H_{21}N_3O_4PF$ 382; found 382. Anal. ($C_{17}H_{21}N_3O_4PF$) C, H, N.

The following compounds were prepared in a similar manner as for compound **4.4**:

4-Amino-1-methyl-2-[2-(5-phosphono)furanyl]benzimidazole (1.1). mp > 230 °C. ^{1}H NMR (D_2O): δ 3.91 (s, 3H), 6.61−7.19 (m, 5H). Anal. ($C_{12}H_{12}N_3O_4P\cdot 1H_2O$) C, H, N.

4-Amino-1-ethyl-2-[2-(5-phosphono)furanyl]benzimidazole (1.2).). ^{1}H NMR (D_2O): δ 0.98 (t, J = 7.4 Hz, 3H), 4.19 (q, J = 7.4 Hz, 2H), 6.71−7.22 (m, 5H); mp > 250 °C. Anal. ($C_{13}H_{14}N_3O_4P\cdot 1H_2O$) C, H, N.

4-Amino-L-propyl-2-[2-(5-phosphono)furanyl]benzimidazole (1.3). ^{1}H NMR (D_2O): δ 0.78 (t, J = 7.2 Hz, 3H), 1.78 (m, 2H), 4.39 (t, J = 7.2 Hz, 2H), 6.61−7.21 (m, 5H); mp > 230 °C. Anal. ($C_{14}H_{16}N_3O_4P + 1.25H_2O$) C, H, N.

4-Amino-l-isobutyl-2-[2-(5-phosphono)furanyl]benzimidazole (1.4). ^{1}H NMR (CD_3OD): δ 0.71 (d, J = 7.2 Hz, 6H), 2.01 (m, 1H), 4.07 (d, J = 7.2 Hz, 2H), 6.71−7.09 (m, 5H). Anal. ($C_{15}H_{18}N_3O_4P\cdot 1.9H_2O$) C, H, N.

4-Amino-1-cyclopropylmethyl-2-[2-(5-phosphono)furanyl]benzimidazole (1.5). mp > 230 °C. ^{1}H NMR (D_2O): δ 0.22 (m, 2H), 0.95 (m, 2H), 1.25 (m, 1H), 4.26 (d, J = 7.0 Hz, 2H), 6.76−7.31 (m, 5H). Anal. ($C_{15}H_{16}N_3O_4P\cdot 0.75CH_2Cl_2$) C, H, N.

4-Amino-1-cyclobutylmethyl-2-[2-(5-phosphono)furanyl]benzimidazole (1.6). mp > 230 °C. ^{1}H NMR (D_2O): δ 1.61−1.88 (m, 6H), 2.78 (m, 1H), 4.42 (d, J = 7.0 Hz, 2H), 6.62−7.19 (m, 5H). Anal. ($C_{16}H_{18}N_3O_4P\cdot 0.5H_2O$) C, H, N.

4-Amino-1-cyclopentylmethyl-2-[2-(5-phosphono)furanyl]benzimidazole (1.7). mp > 230 °C. ^{1}H NMR (D_2O): δ 0.98−1.50 (m, 8H), 2.23 (m, 1H), 4.23 (d, J = 7.2 Hz, 2H), 6.55−7.09 (m, 5H). Anal. ($C_{17}H_{20}N_3O_4P\cdot 1.4H_2O$) C, H, N.

4-Amino-1-cyclohexanemethyl-2-[2-(5-phosphono)furanyl]benzimidazole (1.8). mp 210 °C. ^{1}H NMR (D_2O): δ 0.98−1.85 (m, 11H), 4.21 (d, J = 7.3 Hz, 2H), 6.60−7.15 (m, 5H). Anal. ($C_{18}H_{22}N_3O_4P\cdot 0.5$AcOH) C, H, N.

4-Amino-1-cycloheptanemethyl-2-[2-(5-phosphono)furanyl]benzimidazole (1.9). mp 198 °C. ^{1}H NMR (D_2O): δ 1.08−1.60 (m, 12H), 2.02 (m, 1H), 4.21 (d, J = 7.2 Hz, 2H), 6.61−7.16 (m, 5H). Anal. ($C_{19}H_{24}N_3O_4P\cdot 0.5H_2O$) C, H, N.

4-Amino-1-norbornylmethyl-2-[2-(5-phosphono)furanyl]benzimidazole (1.10). mp > 230 °C. ^{1}H NMR (D_2O): δ 0.67−2.02 (m, 11H), 4.41 (d, J = 7.4 Hz, 2H), 6.64−7.20 (m, 5H). Anal. ($C_{19}H_{22}N_3O_4P\cdot 0.75H_2O$) C, H. N.

4-Amino-1-benzyl-2-[2-(5-phosphono)furanyl]benzimidazole (1.11). mp > 250 °C. ^{1}H NMR (DMSO-d_6): δ 5.04 (s, 2H), 5.96 (s, 2H),

6.48−7.19 (m, 10H). Anal. ($C_{18}H_{14}N_3O_4PNa_2 \cdot 1.6H_2O$) C, H, N. Mass calcd for $C_{18}H_{16}N_3O_4P$ = 369. Found: MH+ = 370; MH− = 368.

4-Amino-1-(4-*t*-butylbenzyl)-2-[2-(5-phosphono)furanyl]benzimidazole (1.12). mp = 246−249 °C. ^{1}H NMR (DMSO-d_6): δ 1.18 (s, 9H), 5.90 (s, 2H), 6.56−7.26 (m, 11H). Anal. ($C_{22}H_{24}N_3O_4P \cdot 0.3HBr \cdot 0.2PhMe$) C, H, N. Mass calcd for $C_{22}H_{24}N_3O_4P$ = 425. Found: MH+ = 426; MH′ = 424.

4-Amino-1-(3-trifluoromethylbenzyl)-2-[2-(5-phosphono)furanyl]-benzimidazole (1.13). mp 235−239 °C. ^{1}H NMR (DMSO-d_6): δ 5.83 (s, 2H), 6.45−7.69 (m, 11H). Anal. ($C_{19}H_{15}N_3O_4PF_3 \cdot 0.7HBr \cdot 0.6PhMe$) C, H, N.

4-Amino-1-(4-phenylbenzyl)-2-[2-(5-phosphono)furanyl]benzimidazole (1.14). mp >220 °C. ^{1}H NMR (D_2O): δ 5.73 (s, 2H), 6.25−7.59 (m, 14H). Anal. ($C_{24}H_{20}N_3O_4P \cdot 0.66H_2O$) C, H, N.

4-Amino-1-(3-furanylmethyl)-2-[2-(5-phosphono)furanyl]benzimidazole (1.15). mp >230 °C. ^{1}H NMR (D_2O): δ 5.48 (s, 2H), 6.24−7.42 (m, 8H). Mass calc. for $C_{16}H_{14}N_3O_5P$ 358; obsd 358.

4-Amino-1-(3-hydroxybenzyl)-2-[2-(5-phosphono)furanyl]benzimidazole (1.16). mp 232−234 °C. ^{1}H NMR (D_2O): δ 5.87 (s, 2H), 6.43−7.10 (m, 9H). Anal. ($C_{18}H_{16}N_3O_5P \cdot 2H_2O$) C, H, N.

4-Amino-1-(3-thienylmethyl)-2-[2-(5-phosphono)furanyl]benzimidazole (1.17). mp = 200−205 °C. ^{1}H NMR (D_2O): δ 5.86 (s, 2H), 6.67−7.42 (m, 8H). Anal. ($C_{16}H_{14}N_3O_4PS \cdot 1.7H_2O$) C, H, N.

4-Amino-5-ethyl-1-isobutyl-2-[5-(2-phosphono)furanyl]benzimidazole (2.1). mp = 220−225 °C. ^{1}H NMR (DMSO-d_6): δ 0.87 (d, 6H, J = 6.7 Hz), 1.17 (t, 3H, J = 7.3 Hz), 1.9−2.25 (m, 1H), 2.62 (t, 2H, J = 7.3 Hz), 4.29 (d, 1H, J = 7.3 Hz), 6.87 (d, 1H, J = 8.3 Hz), 7.01 (d, 1H, J = 8.3 Hz), 7.1 (m, 1H), 7.2 (m, 1H). Anal. ($C_{17}H_{22}N_3O_4P \cdot 0.5HBr$) C, H, N.

4-Amino-5-propyl-1-isobutyl-2-[5-(2-phosphono)furanyl]benzimidazole (2.2). mp = 207−210 °C. ^{1}H NMR (DMSO-d_6): δ 0.85 (d, 6H, J = 6.6 Hz), 0.92 (t, 3H, J = 7.5 Hz), 1.4−1.7 (m, 2H), 1.95−2.2 (m, 1H), 2.55 (t, 2H, J = 7.0 Hz), 4.27 (d, 1H, J = 7.0 Hz), 6.87 (d, 1H, J = 8.2 Hz), 7.04 (d, 1H, J = 8.3 Hz), 7.09 (m, 2H), 7.21 (m, 1H). Anal. ($C_{18}H_{24}N_3PO_4 \cdot 2H_2O$) C, H, N.

4-Amino-5-methoxy-1-isobutyl-2-[5-(2-phosphono)furanyl]benzimidazole (2.3). mp = 212−213 °C. ^{1}H NMR (D_2O): δ 0.75 (d, J = 7.0 Hz, 6H), 2.09 (m, 1H), 3.80 (s, 3H), 4.18 (d, J = 7.0 Hz, 2H), 6.70−7.08 (m, 4H). Anal. ($C_{16}H_{20}N_3O_5P \cdot H_2O$) C, H, N.

4-Amino-5-chloro-1-isobutyl-2-[2-(5-phosphono)furanyl]benzimidazole (2.5). mp >240 °C. ^{1}H NMR (D_2O): δ 0.71 (d, J = 7.2 Hz, 6H), 2.01 (m, 1H), 4.08 (d, J = 7.2 Hz, 2H), 6.71−7.10 (m, 4H). Anal. ($C_{15}H_{17}ClN_3O_4P \cdot 0.8H_2O$) C, H, N.

4-Amino-7-chloro-1-isobutyl-2-[2-(5-phosphono)furanyl]benzimidazole (2.6). mp >239 °C. ^{1}H NMR (D_2O): δ 0.72 (d, J = 7.0 Hz, 6H), 2.03 (m, 1H), 4.55 (d, J = 7.0 Hz, 2H), 6.51−7.08 (m, 4H). Anal. ($C_{15}H_{17}N_3O_4ClP \cdot 1HBr \cdot 1CH_2Cl_2$) C, H, N.

4-Amino-5-bromo-1-isobutyl-2-[2-(5-phosphono)furanyl]benzimidazole (2.7). mp >239 °C. ^{1}H NMR (D_2O): δ 0.74 (d, J = 7.1 Hz, 6H), 2.05 (m, 1H), 4.13 (d, J = 7.1 Hz, 2H), 6.71−7.29 (m, 4H). Anal. ($C_{15}H_{17}N_3O_4BrP \cdot 0.5H_2O$) C, H, N.

4-Amino-7-bromo-1-isobutyl-2-[2-(5-phosphono)furanyl]benzimidazole (2.8). mp >230 °C. ^{1}H NMR (D_2O): δ 0.75 (d, J = 7.3 Hz, 6H), 2.05 (m, 1H), 4.15 (d, J = 7.3 Hz, 2H), 6.71−7.31 (m, 4H). Anal. ($C_{15}H_{17}N_3O_4BrP \cdot 0.25H_2O$) C, H, N.

4-Amino-5-fluoro-1-isobutyl-2-[5-(2-phosphono)furanyl]benzimidazole (2.9). mp = 230−235 °C. ^{1}H NMR (D_2O): δ 0.84 (d, J = 6.6 Hz, 6H), 2.23 (m, 1H), 4.28 (d, J = 7.4 Hz, 2H), 6.77−7.16 (m, 4H). Anal. ($C_{15}H_{17}N_3O_4PF \cdot 0.8H_2O$) C, H, N.

4-Amino-5-fluoro-1-(2-ethylbutyl)-2-[5-(2-phosphono)furanyl]-benzimisdazole (2.10). mp = 178−182 °C (dec). ^{1}H NMR (DMSO-d_6): δ 0.74 (t, 6H, J = 7.0 Hz), 0.8−1.4 (m, 4H), 1.4−1.8 (m, 1H), 4.33 (d, 2H, J = 7.4 Hz), 6.85 (dd, 1H, J_1 = 8.8, J_2 = 3.5 Hz), 6.9−7.3 (series of m, 3H). Anal. ($C_{17}H_{21}N_3O_4FP \cdot 1.0H_2O$) C, H, N.

4-Amino-5-fluoro-1-(3-pentyl)-2-[5-(2-phosphono)furanyl]benzimidazole (2.11). mp = 180−185 °C (dec). ^{1}H NMR (DMSO-d_6): δ 0.6.3 (t, 6H, J = 7.0 Hz), 1.6−2.3 (series of m, 4H), 4.5 (m, 1H), 6.83 (dd, 1H, J_1 = 8.7, J_2 = 3.6 Hz), 6.9 (dd, 1H, J_1 = 8.7, J_2 = 8.7 Hz), 7.0 (m, 2H). Anal. ($C_{16}H_{19}N_3O_4FP \cdot 1.5H_2O$) C, H, N.

4-Amino-5-fluoro-1-cyclopropylmethyl-2-[5-(2-phosphono)-furanyl]benzimidazole (2.12). mp = 258−260 °C. ^{1}H NMR (DMSO-d_6): δ 0.1−0.4 (m, 4H), 1.35 (m, 1H), 4.58 (d, 2H, J = 7.0 Hz), 6.85−7.11 (m, 4H). Anal. ($C_{15}H_{15}N_3O_4PF \cdot 0.3H_2O$) C, H, N.

4-Amino-5,7-dibromo-1-isobutyl-2-[2-(5-phosphono)furanyl]benzimidazole (3.1). mp >215 °C. ^{1}H NMR (D_2O): δ 0.51 (d, J = 7.0 Hz, 6H), 1.81 (m, 1H), 4.29 (d, J = 7.4 Hz, 2H), 6.71 (m, 1H), 6.83 (m, 1H), 7.29 (s, 1H). Anal. ($C_{15}H_{16}Br_2N_3O_4P$) C, H, N.

4-Amino-5,7-dichloro-1-isobutyl-2-[5-(2-phosphono)furanyl]benzimidazole (3.2). mp = 205−207 °C. ^{1}H NMR (D_2O): δ 0.63 (d, J = 7.0 Hz, 6H), 1.91 (m, 1H), 4.41 (d, J = 7.4 Hz, 2H), 6.72 (m, 1H), 7.01 (m, 2H). Anal. ($C_{15}H_{16}N_3O_4Cl_2P \cdot 0.5H_2O$) C, H, N.

4-Amino-5-fluoro-7-chloro-1-isobutyl-2-[5-(2-phosphono)furanyl]-benzimidazole (3.3). mp = 220−225 °C. ^{1}H NMR (DMSO-d_6): δ 0.76 (d, 6H, J = 6.7 Hz), 1.9−2.1 (m, 2H), 4.58 (d, 2H, J = 7.4 Hz), 7.06 (m, 1H), 7.16 (d, 1H, J = 11.6 Hz), 7.2 (m, 1H). Anal. ($C_{15}H_{16}N_3O_4FClP \cdot 0.9HBr$) C, H, N.

4-Amino-5-fluoro-7-bromo-1-isobutyl-2-[5-(2-phosphono)furanyl]-benzimidazole (3.4). mp = 195−200 °C. ^{1}H NMR (DMSO-d_6): δ 0.77 (d, 6H, J = 6.7 Hz), 1.95−2.15 (m, 2H), 4.62 (d, 2H, J = 7.5 Hz), 7.1 (m, 1H), 7.22 (m, 1H), 7.31 (d, 1H, J = 11.6 Hz). Anal. ($C_{15}H_{16}N_3BrFPO_4$) C, H, N.

4-Amino-5-fluoro-6-chloro-1-isobutyl-2-[5-(2-phosphono)furanyl]-benzimidazole (3.5). mp = 175−180 °C. ^{1}H NMR (DMSO-d_6): δ 0.81 (d, 6H, J = 6.7 Hz), 1.9−2.1 (m, 1H), 4.24 (d, 1H, J = 7.0 Hz), 6.02 (br s, 2H), 6.95 (d, 1H, J = 9.7 Hz), 7.05 (m, 1H), 7.12 (m, 1H). Anal. ($C_{15}H_{16}N_3ClFPO_4 \cdot 2H_2O$) C, H, N.

4-Amino-5-bromo-6,7-dichloro-2-[5-(2-phosphono)furanyl]benzimidazole (3.6). mp = 224−225 °C. ^{1}H NMR (D_2O): δ 0.64 (d, J = 7.1 Hz, 6H), 1.98 (m, 1H), 4.51 (d, J = 7.3 Hz, 2H), 6.72 (m, 1H), 6.96 (m, 1H). Anal. ($C_{15}H_{15}N_3O_4Cl_2BrP \cdot 1HBr \cdot 0.9PhMe$) C, H, N.

4-Amino-7-vinyl-5-fluoro-1-isobutyl-2-[5-(2-phosphono)furanyl]-benzimidazole (3.7). mp = 238−242 °C. ^{1}H NMR (DMSO-d_6): δ 0.83 (d, 6H, J = 6.6 Hz), 1.9−2.1 (m, 1H), 4.6 (d, 2H, J = 6.8 Hz), 5.75 (d, 1H, J = 9.3 Hz), 6.05−7.51 (several m, 5H). Anal. ($C_{17}H_{19}N_3O_4FP \cdot 1.2H_2O$) C, H, N.

4-Amino-7-cyclopropyl-5-fluoro-1-isobutyl-2-[5-(2-phosphono)-furanyl]-benzimidazole (3.8). mp 250−255 °C (dec). ^{1}H NMR (DMSO-d_6): δ 0.6−0.8 (m, 2H, buried under the d), 0.7 (d, 6H, J = 6.7 Hz), 0.85−1.0 (m, 2H), 1.8−2.0 (m, 1H), 2.05−2.3 (m, 1H), 4.67 (d, 2H, J = 7.5 Hz), 6.74 (d, 1H, J = 12.7 Hz), 7.02 (m, 1H), 7.13 (m, 1H). Anal. ($C_{18}H_{21}N_3O_4FP \cdot 0.25\ H_2O$) C, H, N.

4-Amino-7-phenyl-5-fluoro-1-isobutyl-2-[5-(2-phosphono)furanyl]-benzimidazole (4.1). mp 240−241 °C (dec). ^{1}H NMR (CD_3OD + 1 drop of NaOD): δ 0.3 (d, 6H, J = 6.6 Hz), 1.45 (m, 1H), 4.1 (d, 1H, J = 6.8 Hz), 6.9 (m, 2H), 7.08 (m, 1H), 7.5−7.6 (m, 5H). Anal. ($C_{21}H_{21}N_3O_4FP \cdot 0.05H_2O$) C, H, N.

4-Amino-7-(*p*-fluorophenyl)-5-fluoro-1-isobutyl-2-[5-(2-phosphono)furanyl]-benzimidazole (4.2). mp 239−240 °C (dec). ^{1}H NMR (CD_3OD +1 drop of NaOD): δ 0.32 (d, 6H, J = 6.6 Hz), 1.47 (m, 1H), 4.13 (d, 2H, J = 6.7 Hz), 6.8−7.0 (m, 2H), 7.05 (m, 1H), 7.1−7.3 (m, 1H), 7.3−7.6 (m, 3H). Anal. ($C_{21}H_{20}N_3O_4F_2P$) C, H, N.

4-Amino-7-(*p*-chlorophenyl)-5-fluoro-1-isobutyl-2-[5-(2-phosphono)furanyl]-benzimidazole (4.3). mp 235−236 °C (dec). ^{1}H NMR (DMSO-d_6): δ 0.28 (d, 6H, J = 6.6 Hz), 1.3 (m, 1H), 3.97 (d, 2H, J = 6.8 Hz), 6.9−7.6 (series of m, 7H). Anal. ($C_{21}H_{20}N_3O_4FClP$) C, H, N.

4-Amino-5-fluoro-7-(*n*-propyl)-1-isobutyl-2-[5-(2-phosphono)-furanyl]benzimidazole (4.5). mp 220−230 °C (dec). ^{1}H NMR (DMSO-d_6): δ 0.68 (d, 6H, J = 6.6 Hz), 0.92 (t, 3H, J = 7.0 Hz), 1.4−1.7 (m, 2H), 1.8 (m, 1H), 2.7 (t, 2H, J = 6.6 Hz), 4.33 (d, 2H, J = 7.8 Hz), 6.8 (d, 1H, J = 12.8 Hz), 7.0 (m, 1H), 7.11 (m, 1H). Anal. ($C_{19}H_{23}N_3O_4FP \cdot 0.85H_2O$) C, H, N.

4-Amino-7-(3,3-dimethyl-*n*-butyl)-5-fluoro-1-isobutyl-2-[5-(2-phosphono)furanyl]-benzimidazole (4.6). mp 200−205 °C (dec). ^{1}H NMR (DMSO-d_6): δ 0.69 (d, 6H, J = 6.6 Hz), 0.97 (s, 9H), 1.4 (m, 2H), 1.9 (m, 1H), 2.7 (m, 2H), 4.36 (d, 2H, J = 6.8 Hz),

6.8 (d, 1H, J = 13.4 Hz), 7.02 (m, 1H), 7.12 (m, 1H). Anal. ($C_{21}H_{29}FN_3O_4P \cdot 0.75H_2O$) C, H, N.

4-Amino-7-(4-methylpentyl)-5-fluoro-1-isobutyl-2-[5-(2-phosphono)furanyl]-benzimidazole (4.7). mp 185−195 °C (dec). ^{1}H NMR (DMSO-d_6): δ 0.68 (d, 6H, J = 6.6 Hz), 0.85 (d, 6H, J = 6.6 Hz), 1.0−2.0 (series of m, 6H), 2.72 (m, 2H), 4.34 (d, 2H, J = 7.2 Hz), 6.8 (d, 1H, J = 12.4 Hz), 7.02 (m, 1H), 7.12 (m, 1H). Anal. ($C_{21}H_{29}N_3O_4FP \cdot 0.25H_2O$) C, H, N.

4-Amino-7-(3-hydroxypropyl)-5-fluoro-1-isobutyl-2-[5-(2-phosphono)furanyl]-benzimidazole (4.8). mp 170−173 °C. ^{1}H NMR (DMSO-d_6): δ 0.68 (d, 6H, J = 6.6 Hz), 1.7−2.0 (m, 3H), 2.83 (t, 2H, J = 7.4 Hz), 3.45 (t, 2H, J = 6.0 Hz), 4.39 (d, 2H, J = 7.8 Hz), 6.8 (d, 1H, J = 12.8 Hz), 7.0 (m, 1H), 7.12 (m, 1H). Anal. ($C_{19}H_{23}N_3O_5FP \cdot 1H_2O$) C, H, N.

4-Amino-5-fluoro-7-[3-(*N*,*N*-dimethylamino)propyl]-1-isobutyl-2-[5-(2-phosphono)-furanyl]benzimidazole (4.9). mp 208−212 °C (dec). ^{1}H NMR (DMSO-d_6): δ 0.71 (d, 6H, J = 6.6 Hz), 1.6−2.0 (m, 3H), 2.7 (br s, 6H), 2.8 (m, 2H), 3.0−3.2 (m, 2H), 4.34 (d, 2H, J = 7.8 Hz), 6.85 (d, 1H, J = 12.8 Hz), 7.05 (m, 1H), 7.1 (m, 1H). Anal. ($C_{20}H_{28}N_4O_4FP \cdot 1HBr \cdot 2H_2O$) C, H, N.

4-Amino-5-fluoro-7-(4-chlorobutyl)-1-isobutyl-2-[5-(2-phosphono)-furanyl]-benzimidazole (4.10). mp 210−220 °C (dec). ^{1}H NMR (DMSO-d_6): δ 0.69 (d, 6H, J = 6.6 Hz), 1.6−2.0 (m, 5H), 2.8 (m, 2H), 3.67 (t, 2H, J = 5.6 Hz), 4.34 (d, 2H, J = 7.8 Hz), 6.83 (d, 1H, J = 12.8 Hz), 7.0 (m, 1H), 7.12 (m, 1H). Anal. ($C_{19}H_{24}N_3O_4FClP \cdot 0.25H_2O$) C, H, N.

Method b. Boron Tribromide Mediated Hydrolysis. 4-Amino-5-hydroxy-1-isobutyl-2-[5-(2-phosphono)furanyl]benzimidazole (2.4). A solution of **11a** (200 mg, 0.48 mmol) in anhydrous dichloromethane (5 mL) was treated with a solution of boron tribromide (1 M, 4.8 mL) in dichloromethane at −78 °C. The resulting reaction mixture was allowed to warm to room temperature. After 16 h, the reaction solution was evaporated to dryness, and the residue was treated with water (3 mL). The precipitate was collected via filtration (washed with water and MeOH) and dried under vacuum at 50 °C to give compound **2.4** (20 mg, 12%); mp = 206−209 °C. ^{1}H NMR (D_2O): δ 0.83 (d, J = 7.0 Hz, 6H), 2.08 (m, 1H), 4.26 (d, J = 7.0 Hz, 2H), 6.67−7.25 (m, 4H). Anal. ($C_{15}H_{18}N_3O_5P \cdot 2.7H_2O$) C, H, N.

General Procedure for the Synthesis of Phosphonate Prodrugs via Alkylation Reactions. 4-Amino-5-fluoro-7-ethyl-1-isobutyl-2-[5-(2-bispivaloyloxymethyl-phosphono)furanyl]benzimidazole (5.1). A suspension of **4.4** (400 mg, 1.05 mmol) in anhydrous acetonitrile (5 mL) was treated with Hunig's base (1.1 mL, 6.3 mmol) and iodomethyl pivolate (2.54 g, 10.5 mmol) at 0 °C under nitrogen. The resulting reaction solution was stirred at 0 °C for 48 h. The reaction solution was evaporated, and the resulting syrup was purified by flash chromatography (SiO_2, 3 cm × 15 cm, 50, 70, 90% EtOAc−hexane, gradient elution) to give **5.1** as a yellow solid (240 mg, 37%). ^{1}H NMR ($CDCl_3$): δ 0.80 (d, 6H, J = 6.6 Hz), 0.93−1.26 (m, 21H), 1.9−2.1 (m, 1H), 2.84 (q, 2H, J = 7.0 Hz), 4.43 (d, 2H, J = 7.0 Hz), 5.51 (bs, 2H), 5.63−5.85 (m, 4H), 6.72−7.38 (m, 3H). Anal. ($C_{29}H_{41}N_3O_8PF$) C, H, N.

4-Amino-5-fluoro-7-ethyl-1-isobutyl-2-[5-(2-bispivaloyloxymethylphosphono)-furanyl]benzimidazole Hydrogenchloride Salt (5.2). A solution of **5.1** (50 mg, 1.05 mmol) in anhydrous benzene (5 mL) was treated with a solution of hydrogenchloride in dioxane (1 N, 0.16 mL) at room temperature. After 10 min, the reaction solution was evaporated and the resulting syrup was crystallized from hexane to give **5.2** as a yellow solid (30 mg, 57%); mp 100−104 °C. ^{1}H NMR ($CDCl_3$): δ 0.71−1.32 (m, 24H), 1.9−2.1 (m, 1H), 2.94 (m, 2H), 3.53 (bs, 2H), 4.47 (m, 2H), 5.72−5.82 (m, 4H), 6.86−7.3 (m, 3H). Anal. ($C_{29}H_{42}N_3O_8PFCl$) C, H, N, F, Cl, P.

4-Amino-5-fluoro-7-ethyl-1-isobutyl-2-{5-[2-(bis(ethyloxycarbonyloxymethyl)-phosphono]furanyl}benzimidazole (5.3). This compound was prepared in a similar manner as **5.1** from compound **4.4**. ^{1}H NMR (DMSO-d_6): δ 0.81 (d, 6H, J = 6.6 Hz), 1.29 (m, 9H), 1.99 (m, 1H), 2.89 (q, 2H, J = 7.0 Hz), 4.21 (q, 4H, J = 7.0 Hz), 4.45 (d, 1H, J = 7.8 Hz), 5.55 (bs, 2H), 5.77 (s, 2H), 5.84 (s, 2H), 6.82−7.35 (m, 3H). Anal. ($C_{25}H_{33}N_3O_{10}FP$) C, H, N.

General Procedure for the Synthesis of Phosphonate Prodrugs via Phosphonic Dichloride Coupling Reactions. 4-Amino-5-fluoro-7-ethyl-1-isobutyl-2-{5-[2-bis(5-methyl-2-thione-1,3-dioxolen-4-yl)methylphosphono]furanyl}benzimidazole (5.6). A suspension of **4.4** (200 mg, 0.52 mmol) in thionyl chloride (1 mL) was heated to reflux for 1 h. The cooled reaction solution was evaporated to dryness. The residue was dissolved in anhydrous chloroform (1.5 mL), cooled to 0 °C, and treated with a solution of 4-hydroxymethyl-5-methyl-[1,3]dioxole-2-thione[25] (306 mg, 2.08 mmol) and pyridine (0.17 mL, 2.08 mmol) in chloroform (1.5 mL) under nitrogen. The resulting reaction solution was stirred at room temperature for 16 h. The reaction solution was evaporated to dryness, and the residue was purified by flash chromatography (SiO_2, 2 cm × 15 cm, 50, 70, 90% EtOAc−hexane, gradient elution) to give **5.6** as a yellow solid (30 mg, 9%). ^{1}H NMR (DMSO-d_6): δ 0.82 (d, 6H, J = 6.6 Hz), 1.31 (t, J = 7.0 Hz, 3H), 1.99 (m, 1H), 2.23 (s, 6H), 2.89 (q, 2H, J = 7.0 Hz), 4.21 (bs, 2H), 4.41 (q, 2H, J = 7.0 Hz), 4.97 (m, 4H), 6.81−7.38 (m, 3H). Anal. ($C_{27}H_{29}N_3O_8PS_2F \cdot 0.25H_2O$) C, H, N.

4-Amino-5-fluoro-7-ethyl-1-isobutyl-2-{5-[2-bis(acetylthioethyl)phosphono]-furanyl}benzimidazole (5.4). This compound was prepared in a similar manner as compound **5.6** from compound **4.4** and 2-acetylthioethanol. ^{1}H NMR ($CDCl_3$): δ 0.84 (d, 6H, J = 6.6 Hz), 1.32 (t, J = 7.2 Hz, 3H), 2.01 (m, 1H), 2.34 (s, 6H), 2.91 (q, 2H, J = 7.0 Hz), 3.21 (t, J = 6.6 Hz, 4H), 4.22 (m, 4H), 4.49 (d, J = 7.0 Hz, 2H), 5.31 (bs, 2H), 6.85 (m, 1H), 6.91 (m, 1H), 7.32 (m, 1H). Anal. ($C_{25}H_{33}N_3O_6PFS_2 \cdot 0.2CH_2Cl_2 \cdot 0.1PhMe$) C, H, N.

4-Amino-7-ethyl-5-fluoro-1-isobutyl-2-{2-[5-bis(4-trimethylacetoxybenzyl)-phosphono]furanyl}benzimidazole (5.5). This compound was prepared in a similar manner as compound **5.6** from compound **4.4** and 4-trimethylacetoxybenzyl alcohol.[26] ^{1}H NMR ($CDCl_3$): δ 0.68 (d, 6H, J = 6.6 Hz), 1.31 (m, 21H), 1.91 (m, 1H), 2.81 (q, 2H, J = 7.0 Hz), 4.39 (d, J = 7.0 Hz, 2H), 5.11 (m, 4H), 6.80−7.38 (m, 13H). Anal. ($C_{41}H_{49}FN_3O_8P \cdot 1.2H_2O$) C, H, N.

4-Amino-7-ethyl-5-fluoro-1-isobutyl-2-[5-({*N*,*N*′-(1-(*S*)-ethoxycarbonyl)ethyl}-phosphonodiamido)furanyl]benzimidazole (5.7). This compound was prepared in a similar manner as compound **5.6** from compound **4.4** (500 mg, 1.3 mmol) and L-alanine ethyl ester to give **5.7** as an orange oil (410 mg, 54%).[26] ^{1}H NMR (DMSO-d_6): δ 0.70 (d, 6H, J = 6.6 Hz), 1.10−1.32 (m, 15H), 1.81 (m, 1H), 2.81 (q, 2H, J = 7.0 Hz), 3.81−4.04 (m, 6H), 4.39 (d, J = 7.0 Hz, 2H), 5.04 (bs, 2H), 5.10−5.31 (m, 2H), 6.89−7.22 (m, 3H). Anal. ($C_{27}H_{39}FN_5O_6P$) C, H, N.

4-Amino-7-ethyl-5-fluoro-1-isobutyl-2-{2-[5-(*O*-ethoxycarbonylmethyl-*N*-(1-methyl-1-ethoxycarbonyl)ethyl)phosphonamido]-furanyl}benzimidazole (5.8). This compound was prepared in a similar manner as compound **5.6** from compound **4.4** (300 mg, 0.79 mmol) by first reacting the phosphonic dichloride with ethyl glycolate (1.0 equiv) and then with L-alanine ethyl ester (2.0 equiv) to give **5.8** as a yellow oil (70 mg, 15%). ^{1}H NMR ($CDCl_3$): δ 0.80 (d, 6H, J = 6.6 Hz), 1.22−1.61 (m, 12H), 1.98 (m, 1H), 2.88 (q, 2H, J = 7.0 Hz), 4.12−4.91 (m, 12H), 6.83 (d, J = 12.8 Hz, 1H), 5.31 (bs, 2H), 7.26 (m, 2H). Anal. ($C_{27}H_{38}FN_4O_7P \cdot 0.1CH_2Cl_2$) C, H, N.

Biology Methods. FBPase enzyme assay, cellular glucose production in rat hepatocyte assay, and glucose lowering in Sprague−Dawley rats assay were carried out as reported before.[3,11,12]

Glucose Lowering in Zucker Diabetic Fatty Rats. ZDF rats were purchased from the Genetic Models Division of Charles-Rivers (Indianapolis, IN) at the age of 10 weeks and allowed to acclimate for 2 weeks prior to evaluation. Animals (n = 6) were housed two per cage under a 12 h lighting cycle (7 a.m.−7 p.m. light) and controlled temperature (22 °C). They were fed Purina 5008 rat chow and drinking water ad libitum unless otherwise

noted. On the morning of the experiment (7 a.m.), catheters were inserted into the tail artery and vein for blood sampling and drug administration, respectively. Rats were housed individually in modified cages, which restricted access to the tail catheter sites, and food was withheld for the remainder of the experiment. At 11 a.m., baseline blood glucose was measured from blood obtained from the tail artery and an infusion of **4.4** at 10 mg/kg/h or saline was initiated. Blood glucose was measured hourly. **4.4** was dissolved at a final concentration of 10 mg/mL in water pH adjusted to 7.4 with 5 N NaOH.

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Supporting Information Available: Elemental analysis data for all final compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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