MB06322 (CS-917): A potent and selective inhibitor of fructose 1,6-bisphosphatase for controlling gluconeogenesis in type 2 diabetes


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In type 2 diabetes, the liver produces excessive amounts of glucose through the gluconeogenesis (GNG) pathway and consequently is partly responsible for the elevated glucose levels characteristic of the disease. In an effort to find safe and efficacious GNG inhibitors, we targeted the AMP binding site of fructose 1,6-bisphosphatase (FBPase). The hydrophilic nature of AMP binding sites and their widespread use for allosteric regulation of enzymes in metabolic pathways has historically made discovery of AMP mimetics suitable for drug development difficult. By using a structure-based drug design strategy, we discovered a series of compounds that mimic AMP but bear little structural resemblance. The lead compound, MB05032, exhibited high potency and specificity for human FBPase. Oral delivery of MB05032 was achieved by using the bisamidate prodrg MB06322 (CS-917), which is converted to MB05032 in two steps through the action of an esterase and a phosphoramidase. MB06322 inhibited glucose production from a variety of GNG substrates in rat hepatocytes and from bicarbonate in male Zucker diabetic fatty rats. Analysis of liver GNG pathway intermediates in Zucker diabetic fatty rats after oral administration indicated improved liver GNG. MB06322 to Zucker diabetic fatty rats led to a dose-dependent decrease in plasma glucose levels independent of insulin levels and nutritional status. Glucose lowering occurred without signs of hypoglycemia or significant elevations in plasma lactate or triglyceride levels. The findings suggest that potent and specific FBPase inhibitors represent a drug class with potential to treat type 2 diabetes through inhibition of GNG.

The liver (1, 2) and, to a lesser extent, the kidneys (3) are the primary organs responsible for endogenous glucose production (EGP). In type 2 diabetes mellitus (T2DM), excessive EGP in the fasted state and fed state contributes to the chronic elevation of blood glucose levels found in patients with advanced (4, 5) and mild diabetes (6, 7). Moreover, fasting plasma glucose levels correlate with EGP rates in patients with fasting plasma glucose >180 mg/dl (10 mM) (8) and possibly in patients with lower levels (6, 7). Studies using 13C NMR spectroscopy (9) as well as more recent studies using deuterated water (7, 10) attribute the excessive EGP in T2DM patients to increased flux through the gluconeogenesis (GNG) pathway.

Efforts over the past 40 years to discover inhibitors of GNG produced few safe and effective drug candidates (11). Metformin, the only marketed drug that acts, at least partially, through inhibition of GNG (12), inhibits GNG indirectly and only 33–36% at the rarely prescribed maximal human dose (13). Direct GNG inhibitors show more pronounced glucose lowering in animals but not without eliciting safety-related concerns. Hypoglycemia, lactic acidosis, and hypertriglyceridemia are the principle safety risks associated with GNG inhibition as highlighted in studies with phosphoenolpyruvate carboxykinase inhibitors (14), glucose 6-phosphatase inhibitors (15), and transgenic mice overexpressing 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (16).

Our program targets fructose 1,6-bisphosphatase (FBPase; Δ-fructose 1,6-bisphosphate 1-phosphohydrolase, EC 3.1.3.11), a highly regulated, rate-limiting enzyme that catalyzes the second to last step in GNG (17). The attractiveness of FBPase as a drug target is largely based on its position in the GNG pathway, which enables inhibition of GNG from all GNG substrates while avoiding direct effects on glycogenolysis, glycolysis, and the tricarboxylic acid cycle. Furthermore, the near normal clinical profile of patients genetically deficient in FBPase who manage their diet and avoid prolonged fasting (18) suggests that FBPase inhibitors may exhibit an adequate safety margin.

FBPase is naturally inhibited under certain conditions by the substrate analogue fructose 2,6-bisphosphate (Fru 2,6-P2) (19) and by AMP (IC50 = 1 μM), which acts through an allosteric binding site (20). We focused on the AMP binding site largely because we anticipated difficulties in discovering FBPase inhibitors that bind to the highly hydrophilic substrate binding site with sufficient affinity to compete with the buildup of intracellular fructose 1,6-bisphosphate. Targeting the AMP binding site is associated with its own set of well recognized design challenges arising from the hydrophilic nature of AMP sites, their reliance on the negatively charged phosphate group of AMP for binding affinity (21), and the abundance of AMP-binding enzymes controlling key biosynthetic pathways. Previous efforts targeting the AMP site identified several weak FBPase inhibitors (22), including the nonspecific inhibitor aminoimidazole-4-carboxamide riboside monophosphate (ZMP) (23, 24), which lowered glucose and raised lactate levels after reaching millimolar liver levels following systemic administration of aminoimidazole-4-carboxamide riboside to normal mice at high doses (23).

Here, we present our discovery of a series of potent and selective inhibitors of FBPase and the ability of the lead compound, MB06322 (CS-917), to dose-dependently inhibit GNG as well as lower basal and postprandial blood glucose levels in Zucker diabetic fatty (ZDF) rats after oral administration.

Materials and Methods

MB05032, [5-{[2-amino-5-(2-methylpropyl)-4-thiazolyl]-2-furany]phosphonic acid and MB06322 (CS-917), l-alanine, N,N’-{[5-{[2-amino-5-(2-methylpropyl)-4-thiazolyl]-2-furanyl]phosphinylidene}bis-, diethyl ester were synthesized at Metabasis Therapeutics (San Diego). Fru 2,6-P2 and ZMP were purchased from Sigma–Aldrich.

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Abbreviations: GNG, gluconeogenesis; FBPase, fructose 1,6-bisphosphatase; ZDF, Zucker diabetic fatty; T2DM, type 2 diabetes mellitus; PFG, fasting plasma glucose; ZMP, aminoimidazole-4-carboxamide riboside monophosphate; Fru 2,6-P2, fructose 2,6-bisphosphate.

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Purification of FBPase. The pET3a expression vector containing the human liver FBPase gene was a gift from M. Raafat El-Maghrabi (State University of New York, Stony Brook). The enzyme was expressed in Escherichia coli and purified to homogeneity as described in ref. 25. FBPase was partially purified from male Sprague–Dawley rat (Harlan Laboratories) liver and from human muscle tissue (Analytical Biological Services, Wilmington, DE) by using the procedure referenced above through the first Sephadex G-50 column chromatography step.

Enzyme Assays. FBPase activity was measured spectrophotometrically in reactions that coupled the production of fructose 6-phosphate to the reduction of NADP+ (26). AMP-activated protein kinase (rat liver, Upstate Biotechnology, Rochester, NY) was assayed by using the enzyme substrate SAMS according to the supplier’s instructions. AMP deaminase (porcine heart) was purified and assayed as described in ref. 21. Glycogen phosphorylase (rabbit muscle), phosphofructokinase (rabbit liver), and adenylate kinase (rabbit muscle) were obtained from Sigma–Aldrich and assayed as described in refs. 27–29. Kinetic parameters were calculated by means of four-parameter logistics regression with use of SIGMAPLOT 2000 software (Systat, Richmond, CA).

GNG Assays. Hepatocytes were prepared from male Sprague–Dawley rats that were fasted for 24 h or from freely feeding male ZDF rats (Genetics Models, Indianapolis, or Charles River Laboratories) according to the procedure of Berry and Friend (30) as modified by Groen et al. (31). Primary human hepatocytes were obtained from Tissue Transformation Technologies (Edison, NJ). Hepatocytes (10–60 mg/ml) were preincubated with MB06322 or MB05032 in suspension culture for 15–30 min before a 30- to 60-min incubation with gluconeogenic substrate(s). Reactions were terminated by centrifugation. Glucose in cell supernatants was assayed by using a glucose oxidase kit (Sigma) or, in cases for which [14C]lactate [specific activity, 60 mCi/mmol (1 Ci = 37 GBq); Amersham Pharmacia Biosciences] was used, by scintillation counting after deproteinization and batch ion-exchange chromatography (32). Cellular viability was assessed by Trypan blue exclusion.

Male ZDF rats (10–12 weeks old) were instrumented with tail vein catheters and fasted for 6 h. MB06322 or vehicle (polyethylene glycol 400) was then administered orally by gavage at 1 p.m. and followed 2 h later by an i.v. bolus of [14C]bicarbonate (0.4 μCi per g of body weight; Moravek, Brea, CA). Twenty minutes after radiolabel administration, animals were anesthetized with isoflurane, and blood samples were taken from the inferior vena cava. [14C]Glucose was analyzed in the samples as described above.

GNG Intermediates and Precursors. Livers were isolated from male ZDF rats (10 weeks old) that were fasted for 6 h and then treated orally with vehicle (polyethylene glycol 400) or MB06322. Four hours later, animals were anesthetized, and liver biopsies were obtained. Snap-frozen liver samples were extracted in 10% perchloric acid, neutralized, and then analyzed for intermediates and precursors of GNG by means of enzyme-coupled spectrophotometric assays as described in ref. 33. Coupling enzymes for these determinations were obtained from Roche Diagnostics and Sigma.

Acute Oral Studies in ZDF Rats. For the dose–response and time course studies, 8- to 9-week-old or 12- to 13-week-old male ZDF rats were randomized into groups of eight animals and dosed orally with MB06322 or vehicle (polyethylene glycol 400) at 10 a.m. Food was removed at this time and withheld throughout the study. Blood samples were obtained from a tail vein at baseline and at regular time intervals for up to 10 h thereafter. Blood glucose was determined by means of an analyzer (Hemocue, Mission Viejo, CA). Lactate and triglycerides were determined at all time points by using standard assays (Trinity Biotech, St. Louis, and ThermoDMA, Arlington, TX, respectively). Insulin was determined by ELISA (Alpco Diagnostics, Windham, NH). For the glucose tolerance tests, male ZDF rats were fasted for 4.5 h before MB06322 or vehicle administration. A bolus of glucose (2 g/kg) was administered orally 1.5 h later. Blood samples were taken from the tail vein and analyzed for glucose, lactate, and insulin as described above.

Animal Care. Rats were housed under standard vivarium conditions (12-h light/dark cycle) with free access to Purina 5008 (ZDF rats) or standard chow (Sprague–Dawley rats) and water unless otherwise indicated. All studies were conducted in accordance with the Institutional Animal Care and Use Committee guidelines.

Data Analysis. Results are expressed as mean ± standard errors of the mean for all values unless otherwise indicated. Differences between animal groups were evaluated by using ANOVA with Dunnett’s post hoc analysis or the unpaired Student t test where appropriate and are considered significant when P < 0.05.

Results
Design of MB05032. Our initial efforts to identify an AMP mimetic with high FBPase inhibitory potency and specificity focused on the replacement of the phosphate moiety because the negatively charged phosphate not only severely limits oral bioavailability and cell penetration but is also rapidly removed in biological fluids by phosphatases. The dephosphorylated compound exhibits a >104-fold loss in inhibitory potency. The loss in potency was consistent with the x-ray structures of the pig and human FBPase–AMP complexes (34, 25), which showed the phosphate forming a full complement of hydrogen bonds with the protein. Efforts to replace the phosphate group with a carboxylate or other phosphate mimetics consistently led to near complete loss in binding affinity, which likely reflected the binding site architecture and its preference for a negatively charged tetrahedral group. Even the corresponding phosphonate analogue of AMP, i.e., AMP with its 5′ oxygen replaced with a methylene, exhibited a 2,000-fold loss in inhibitor potency, possibly reflecting the importance of the hydrogen bond between 113Tyr and the 5′ oxygen (25).

Successful redesign of AMP was ultimately achieved through insights gained by solving and analyzing 10 2.3- to 2.5-Å resolution x-ray structures of FBPase-inhibitor complexes and by conducting numerous free energy perturbation calculations (35, 36). Analysis of the FBPase–AMP complex showed that the AMP binding conformation had a glycosyl bond torsion angle (O4′-C1′-N9-C4′) of −155.1°, meaning that the C8–H bond of the purine base pointed directly at the phosphate group and, therefore, that the phosphate binding site was accessible from the purine base (Fig. L4). The distance between C8 and the phosphorus atom was 4.24 Å, suggesting that a three-atom spacer inserted between the base and a phosphonic acid moiety might optimally align both for interactions with the binding site (Fig. 1A). Synthesis of adenosine analogues containing a phosphonic acid linked to C8 through various molecular fragments led to the identification of the spacer, 2,5-furanyl, and the initial lead inhibitor series (IC50 ≈ 50–100 μM). Next, the ribosyl group, which forms few strong interactions with FBPase (35), especially in C8-substituted analogues, was replaced by arylalkyl and alkyl groups to enhance interactions with a nearby hydrophobic surface in the binding site cavity derived from the side chains of 177Met, 160Val, 30Leu, and 24Ala. These changes led to a 100-fold improvement in potency.

Efforts to maximize AMP site specificity exploited differences
in binding site interaction patterns derived from analysis of x-ray structures of 25 nucleotide-binding enzymes. Hydrogen bonds formed between the proteins and purine base nitrogens N7, 6NH2, N1, and N3 were found in 26%, 12% (two bonds), 54%, and 43% of the structures, respectively, whereas ligand-scanning results (35) showed that only N7 and the 6NH2 group of AMP formed strong hydrogen bonds with FBPase. These results suggested that replacement of some or all of the nitrogens in the pyrimidine portion of the purine base with CH or removal of the pyrimidine base altogether might improve FBPase binding affinity by decreasing desolvation costs. FBPase specificity was also expected to improve, because hydrogen bond interactions with the pyrimidine base often contribute to AMP binding affinity with other AMP-binding enzymes. By using these concepts, we discovered MB05032 (Fig. 1B), a disubstituted 2-aminooxazole that functions as an AMP mimetic despite lacking all of the structural features characteristic of AMP, i.e., the adenine base and the ribose 5’-phosphate.

**FBPase Inhibition and Specificity.** MB05032 inhibits human liver FBPase with a potency (IC50 = 16 ± 1.5 nM) significantly greater than the natural inhibitor, AMP (IC50 = 1 μM), and the most well characterized AMP mimetic, ZMP (IC50 = 12 ± 1.4 μM) (Fig. 2 and Table 1). Like AMP, MB05032 exhibits competitive kinetics (P.D.v.P., unpublished data) and achieves maximal inhibition (99%) over a narrow concentration range (Fig. 2). MB05032 displaces [14C]AMP bound to FBPase in a concentration-dependent manner confirming the AMP site as the site of interaction (J. Stebbins, personal communication). Addition of Fru 2,6-P2 results in a potentiation of the inhibition as previously reported for AMP (19). MB05032 inhibits rat FBPase 3-fold weaker (IC50 of 61 ± 4 nM) than human FBPase (Table 1), whereas AMP is 20-fold weaker as an inhibitor. Unlike AMP and ZMP, MB05032 is inactive (EC50 > 100 μM) against glycogen phosphorylase and AMP-activated protein kinase, two AMP-binding enzymes controlling carbohydrate metabolism known to be affected by ZMP (EC50 = 110 ± 10 and 82.5 ± 9 μM, respectively). Like AMP, however, MB05032 is a potent inhibitor of human muscle FBPase (IC50 = 29 ± 1 nM), an isoenzyme derived from a separate gene that has 77% amino acid identity to the liver enzyme (37).

**Design of MB06322.** MB05032, like most phosphonic acids, exhibits low oral bioavailability in animals (<2%) (data not shown). Accordingly, a large set of known phosphonic acid prodrugs (38) and novel prodrugs (39) were prepared and extensively evaluated in an effort to find a prodrug with improved oral bioavailability, good aqueous stability, and no risk of prodrug byproduct-related toxicity. These efforts led to the discovery of a bisamidate prodrug series, which unlike other phosphate prodrugs, had all of the desired properties. The lead prodrug, MB06322, is stable in aqueous solutions (t50 > 7 days at pH 3.0–7.4), but cleaves rapidly to MB05032 and the nontoxic byproducts alanine and ethanol in the presence of rat and human liver S9 fractions. Prodrug cleavage begins with an esterase-catalyzed reaction to generate a monoamidate intermediate (MB06633, Fig. 3A) followed by a phosphoramidase-mediated reaction analogous to that described for aryl phosphoramidate prodrugs (40). Oral administration of MB06322 to rats resulted in rapid absorption and conversion to MB05032 and satisfactory oral bioavailability (>20%).

**Glucose Production in Hepatocytes.** MB06322 and MB05032 exhibit concentration-dependent inhibition of glucose production from all common GNG precursors in rat and human hepatocytes (Table 2). As shown for human hepatocytes in Fig. 3B, MB06322 undergoes rapid intracellular conversion to the monoamidate intermediate MB06633 and then to MB05032. MB06322 inhibits glucose synthesis by human hepatocytes over a narrow concentration range with full inhibition achieved at 1 μM (Fig. 3C). Relative to MB06322, MB05032 is a 5- and 226-fold less potent prodrug of MB06322.

**Table 1. FBPase inhibitory potency and specificity**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>MB05032 EC50, nM</th>
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<tbody>
<tr>
<td>Human liver FBPase</td>
<td>16 ± 1.5</td>
</tr>
<tr>
<td>Human muscle FBPase</td>
<td>29 ± 1</td>
</tr>
<tr>
<td>Rat liver FBPase</td>
<td>61 ± 14</td>
</tr>
<tr>
<td>Human erythrocyte AMP deaminase</td>
<td>&gt;100,000</td>
</tr>
<tr>
<td>Rabbit muscle glycogen phosphorylase</td>
<td>&gt;100,000</td>
</tr>
<tr>
<td>Human AMP-activated protein kinase</td>
<td>&gt;100,000</td>
</tr>
<tr>
<td>Rabbit muscle adenylate kinase</td>
<td>&gt;500,000</td>
</tr>
<tr>
<td>Rabbit liver phosphofructokinase</td>
<td>&gt;100,000</td>
</tr>
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EC50 values were determined as described in Materials and Methods.
inhibitor of glucose synthesis by rat and human hepatocytes, respectively, suggesting that the negatively charged phosphonic acid impedes entry into hepatocytes.

**Inhibition of GNG in ZDF Rats.** Oral administration of MB06322 to fasted male ZDF rats results in dose-dependent inhibition of $^{14}$Cbicarbonate incorporation into glucose (Fig. 4A). Maximal GNG inhibition ($\sim 80\%$) is achieved by using oral MB06322 doses of 100–300 mg/kg. Significant glucose lowering is observed at 30 mg/kg, which suggests that GNG inhibition must be $>15\%$ to lower basal glucose levels. Measurement of GNG pathway precursors, intermediates, and products in the liver after oral administration of MB06322 confirmed FBPase as the target enzyme. As shown in Fig. 4B, intermediates upstream of FBPase are elevated 1.5- to 3.1-fold in MB06322-treated rats relative to vehicle-treated rats, whereas glucose 6-phosphate and glucose are unchanged and 41% decreased, respectively.

**Glucose Lowering in ZDF Rats.** Oral administration of MB06322 to young (8–9 weeks old) ZDF rats with mild diabetes (basal insulin levels of $7.7 \pm 0.7\, \text{ng/ml}$ and aged (12–13 weeks) ZDF rats with overt diabetes (basal insulin levels of $0.65 \pm 0.16\, \text{ng/ml}$) results in dose-dependent glucose lowering (Fig. 5A and B). The dose–response is relatively steep, with 6–10 mg/kg and 30–100 mg/kg being the approximate doses associated with minimal and maximal activity, respectively. Glucose lowering occurs rapidly with maximal effects usually apparent 2.5–5 h after drug administration (Fig. 5C and D). No significant differences in glucose levels remain between MB06322-treated and vehicle-treated animals 10 h after dosing, which likely reflects the simultaneous disappearance of MB05032 from the liver and plasma. No increases in blood lactate levels (AUC$_{0-\text{inf}}$) are associated with MB06322 treatment in young ZDF rats whereas lactate levels are increased 72% and 78% in older ZDF rats administered 30 and 100 mg/kg of MB06322, respectively. In addition to reducing basal glucose levels, oral glucose tolerance is improved following oral administration of MB06322 (30 mg/kg). Reductions are evident in young and aged ZDF rats (Fig. 5E and F) with the AUC$_{0-\text{inf}}$ of blood glucose normalized to baseline reduced 65% and 55%, respectively, relative to vehicle-treated animals. Similar to the dose–response studies, MB06322 treatment results in elevated lactate levels (79%) only in aged ZDF rats. No significant differences in insulin or triglyceride levels are detected between vehicle- and drug-treated animals in any study.

**Discussion**

FBPase has long been recognized as a potential drug target for diabetes (23, 11, 22). Efforts over the past 20 years to discover potent and specific FBPase inhibitors by screening large compound libraries yielded few promising leads probably reflecting the known difficulties in finding high-affinity ligands for hydrophobic binding sites (22, 41, 42). Conversely, our program centered on a structure-guided approach, which we used to identify a series of nonnucleoside inhibitors capable of forming a unique set of interactions with the AMP binding site. The lead compound identified through these efforts, MB05032, shares little structural similarity with AMP (Fig. 1). In place of the adenine base and the ribose 5'-phosphate of AMP, MB05032 uses a...
2-aminothiazole substituted with a furanyl phosphonic acid and an alkyl group to bind to the AMP site and form favorable interactions with the base and phosphate binding sites and a nearby hydrophobic region. Binding of MB05032 leads to potent inhibition of FBPase that is synergistic with Fru 2,6-P2, signifying that MB05032 functions as a true AMP mimic. The efficacy and safety profile of MB06322 in the ZDF rat provides the first insight into the therapeutic index of FBPase inhibitors. Hypoglycemia, one of the major safety concerns associated with GNG inhibition, was not observed, even with MB06322 doses 10-fold above the minimum effective dose administered to ZDF rats. The resistance of ZDF rats to hypo-glycemia is presumably related to the availability of hepatic glycogen reserves and possibly some residual GNG activity (7–15%). Elevated lactate and triglyceride levels are two other possible consequences of GNG inhibition that impact the safety potential of FBPase inhibitors. Importantly, neither lactate nor triglycerides increased in 8- to 9-week-old ZDF rats with mild diabetes treated with high doses of MB06322. In ZDF rats with more advanced disease, lactate and triglyceride levels were elevated but only modestly (<2-fold). These results suggest that, unlike inhibitors of other GNG enzymes (14, 15), FBPase inhibitors may lower glucose with an adequate safety margin.

The efficacy and safety profile of MB06322 in the ZDF rat suggests that FBPase inhibitors may represent a class of drugs useful for treating early-stage diabetes in which postprandial hyperglycemia is the primary contributor to the elevated HbA1c levels and late stage diabetes in which fasting hyperglycemia becomes the most important contributor (44). Moreover, the independence of the glucose-lowering effect described here, MB06322, exhibits good stability across a broad pH range, generates non-toxic byproducts, and results in satisfactory oral bioavailability in the rat (>20%). Moreover, the bisamidate series is readily synthesized and, unlike earlier phosphoramidate prodrugs (40), enables the phosphonate moiety to remain achiral.

Exposure of primary rat hepatocytes to MB06322 leads to dose-dependent inhibition of GNG (Fig. 3). Consistent with FBPase as the target enzyme, GNG inhibition is observed in hepatocytes independent of the GNG substrate and at intracellular MB05032 concentrations expected to inhibit rat FBPase. The 24- and 11-fold rightward shift in the EC50 values for rat and human hepatocytes, respectively, relative to the IC50 values for the isolated enzyme reflects the free steady-state intracellular level of MB05032 during the time of the measurement, which further reflects time-dependent conversion of MB06322 to MB05032 and the secretion of MB05032 from the cell. In the ZDF rat, oral administration of MB06322 produces dose-dependent inhibition of GNG as demonstrated by the relative difference in bicarbonate incorporation into glucose between vehicle- and MB06322-treated rats (Fig. 4). The steep inhibition curves observed in both hepatocytes and in vivo are likely a result of the allosteric inhibition mechanism and to some degree the control strength of FBPase (17). Interestingly, in the ZDF rat, maximally effective doses of MB06322 result in ~80–85% inhibition of GNG, which might arise from some residual enzyme activity (1–2%) associated with the FBPase–AMP complex coupled with the buildup of Fru 1,6-P2.

The potential glucose-lowering effects of FBPase inhibitors are evident from studies with ZDF rats administered MB06322 (Fig. 5). Glucose lowering is rapid and occurs over a narrow but similar dose range as observed for GNG inhibition. Importantly, glucose lowering takes place despite the presence of large liver glycogen reserves, suggesting that mechanisms designed to maintain liver glucose production through compensatory increases in glycogen breakdown, i.e., hepatic autoregulation (43), are unable to fully compensate for the decrease in GNG. The robustness of the glucose-lowering effect is highlighted by the efficacy observed in mildly diabetic, hyperinsulinemic ZDF rats and overtly diabetic, hypoinsulinemic ZDF rats. These results suggest that FBPase inhibitors act irrespective of insulin levels. Furthermore, reductions in fasting glucose levels and glucose levels after a glucose load suggest that FBPase inhibitors could provide increased glycemic control throughout the day.

The ZDF rat studies also provide the first insight into the therapeutic index of FBPase inhibitors. Hypoglycemia, one of the major safety concerns associated with GNG inhibition, was not observed, even with MB06322 doses 10-fold above the minimum effective dose administered to ZDF rats. The resistance of ZDF rats to hypoglycemia is presumably related to the availability of hepatic glycogen reserves and possibly some residual GNG activity (7–15%). Elevated lactate and triglyceride levels are two other possible consequences of GNG inhibition that impact the safety potential of FBPase inhibitors. Importantly, neither lactate nor triglycerides increased in 8- to 9-week-old ZDF rats with mild diabetes treated with high doses of MB06322. In ZDF rats with more advanced disease, lactate and triglyceride levels were elevated but only modestly (<2-fold). These results suggest that, unlike inhibitors of other GNG enzymes (14, 15), FBPase inhibitors may lower glucose with an adequate safety margin.

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on insulin levels may differentiate FBPase inhibitors from other drug classes used to treat T2DMs (45) whose effects depend on insulin and consequently wane with time because of progressive β cell dysfunction (46). Last, FBPase inhibitors may inhibit GNG and correspondingly lower glucose more than drugs that indirectly inhibit GNG, because these drugs typically modulate only one of the many factors that contribute to elevated GNG in T2DMs, e.g., hepatic insulin resistance, elevated levels of glucagon, free fatty acids, GNG substrates, and hormones such as cortisol (47).

In summary, a structure-guided drug design strategy led to the discovery of a series of potent and selective FBPase inhibitors. The lead compound, MB06322, administered to ZDF rats resulted in dose-dependent GNG inhibition and pronounced glucose lowering independent of insulin levels and nutritional status. The favorable efficacy and safety shown in these studies suggest that FBPase inhibitors may represent a class of drugs that could prove useful for treating a broad range of T2DMs either alone or in combination with drugs acting predominantly through insulin secretion or insulin action.

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