MLR-1023 Is a Potent and Selective Allosteric Activator of Lyn Kinase In Vitro That Improves Glucose Tolerance In Vivo

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ABSTRACT

2(1H)-pyrimidinone,5-(3-methylphenoxy) (MLR-1023) is a candidate for the treatment of type 2 diabetes. The current studies were aimed at determining the mechanism by which MLR-1023 mediates glycemic control. In these studies, we showed that MLR-1023 reduced blood glucose levels without increasing insulin secretion in vivo. We have further determined that MLR-1023 did not activate peroxisome proliferator-activated α, δ, and γ receptors or glucagon-like peptide-1 receptors or inhibit dipeptidyl peptidase-4 or α-glucosidase enzyme activity. However, in an in vitro broad kinase screen MLR-1023 activated the nonreceptor-linked Src-related tyrosine kinase Lyn. MLR-1023 increased the $V_{max}$ of Lyn with an EC$_{50}$ of 63 nM. This Lyn kinase activation was ATP binding site independent, indicating that MLR-1023 regulated the kinase through an allosteric mechanism. We have established a link between Lyn activation and blood glucose lowering with studies showing that the glucose-lowering effects of MLR-1023 were abolished in Lyn knockout mice, consistent with existing literature linking Lyn kinase and the insulin-signaling pathway. In summary, these studies describe MLR-1023 as a unique blood glucose-lowering agent and show that MLR-1023-mediated blood glucose lowering depends on Lyn kinase activity. These results, coupled with other results (J Pharmacol Exp Ther 342:23–32, 2012), suggest that MLR-1023 and Lyn kinase activation may be a new treatment modality for type 2 diabetes.

Introduction

MLR-1023 [Tolimidone; 2(1H)-pyrimidinone,5-(3-methylphenoxy); see Fig. 1] is a small orally bioavailable compound that was originally developed as an antiulcer therapeutic (Lipinski et al., 1980). MLR-1023 reached phase II clinical trials for this indication, exhibited good pharmacokinetic properties, and was well tolerated in patients when administered for up to 6 weeks (Saporito et al., 2012). In a phenotypic screening platform designed to uncover new therapeutically beneficial activities of small molecules, MLR-1023 administration significantly lowered blood glucose in a mouse oral glucose tolerance test (OGTT), suggesting that it might be useful as a therapeutic for the treatment of type 2 diabetes (T2D) (Saporito et al., 2012).

T2D is a heterogeneous disease of disrupted glucose homeostasis driven by insulin resistance, increased hepatic glucose output, and pancreatic β cell dysfunction and loss (DeFronzo, 2010). Oral drugs approved for the treatment of T2D are heterogeneous in their mechanisms, safety profiles, and tolerability, with their utility limited by side-effect profile and/or limited efficacy (Krentz and Bailey, 2005; DeFronzo, 2010). Among the new drugs in development for T2D are those that target protein kinases and kinase signaling pathways (Tahrani et al., 2011), including selective kinase activators (Sanders et al., 2007; Viollet et al., 2007; Simpson et al., 2009; Eglen and Reisine, 2011). Nearly all small-molecule kinase activators act by binding to non-ATP allosteric binding sites on the target protein (Simpson et al., 2009; Eglen and Reisine, 2011). These allosteric regulatory interactions confer a higher degree of selectivity to kinase activators compared with kinase inhibitors that typically bind to highly conserved ATP binding sites on the protein (Eglen and Reisine, 2011).
Lyn kinase is a Src-related nonreceptor-linked tyrosine kinase that is found in an array of cell types and tissues including smooth muscle, liver, brain, myeloid cells, and adipose tissue (Pertel et al., 2006; Scapini et al., 2009; Yamada et al., 2010; Gibb et al., 2011). Although Lyn kinase has been studied primarily in myeloid lineage cells, there are key data that implicate Lyn kinase as a regulator of insulin signaling and glucose regulation. In adipocytes, Lyn kinase activation leads to a direct tyrosine phosphorylation of insulin receptor substrate-1 (IRS-1) (Muller et al., 2000, 2001b). IRS-1 phosphorylation amplifies the insulin receptor-signaling pathway by increasing glucose transporter type 4 translocation and glucose uptake and utilization (Muller et al., 2000, 2001b, 2005). This link between Lyn kinase activation and therapeutic efficacy in T2D has been noted for the oral diabetes therapeutic glimepiride (Muller, 2000; Muller et al., 2001a). Glimepiride-mediated Lyn kinase activation activity may be responsible for glycemic control activity independent of its insulin-secretagogue activity (Muller, 2000; Muller et al., 2005). However, glimepiride-mediated Lyn kinase activation occurs indirectly through the regulation of lipid rafts and is likely to be nonspecific (Muller et al., 2005). A compound that directly activates Lyn kinase would provide a new therapeutic modality that would increase insulin-signaling events without affecting insulin secretion and promote glucose-lowering effects that would be therapeutically beneficial in T2D.

The present studies were designed to investigate the mechanisms involved in MLR-1023-mediated blood glucose lowering. These studies show that MLR-1023 selectively activated Lyn kinase through an allosteric mechanism in vitro, and Lyn kinase activity is critical for MLR-1023-mediated blood glucose-lowering activity in vivo.

Materials and Methods

Materials. MLR-1023 was synthesized according to previously developed synthetic methods (Lipinski et al., 1980) at the Advanced Synthesis Group (Newark, DE). Metformin was obtained from Sigma-Aldrich (St. Louis, MO). Insulin ELISA kits were obtained from Crystal Chem Inc. (Downers Grove, IL). Aviva glucose test strips and monitors were obtained from Roche Diagnostics (Indianapolis, IN). All other compounds and reagents were obtained from Sigma-Aldrich.

Animals. All experiments were conducted in accordance with the National Institutes of Health regulations for animal care (Institute of Laboratory Animal Resources, 1996) and approved by the Institutional Animal Care and Use Committees.

CD1-ICR male mice (Ace Animals, Boyertown, PA), 8 to 10 weeks of age, were used in studies of baseline glucose, glucose tolerance, and insulin levels. Male and female Lyn kinase knockout mice (129S4-LynFm1Smr/J) and wild-type controls (129S1/SvImJ) were acquired from The Jackson Laboratory (Bar Harbor, ME) (Chan et al., 1997). At the time of study, Lyn kinase knockout mice were phenotypically similar to wild-type mice. Mice were fed a standard diet and were 8 weeks of age at the time of study. Mice were housed two to four per cage and given free access to food and water unless otherwise noted. Animals were fed Harlan Chow (2016 Teklad Global 16% Protein Rodent Diet; Harlan, Indianapolis, IN).

Drug Administration. MLR-1023 was formulated in 0.5% carboxymethyl cellulose and 0.025% Tween 80 in phosphate-buffered saline. MLR-1023 was administered intraperitoneally at dose volumes of 5 to 10 ml/kg.

Oral Glucose Tolerance Test and Glucose Measurements. For OGTT studies, mice were fasted for 18 h, treated with drug or vehicle, and then given a glucose solution (1.5 g/kg) via oral gavage. Blood glucose levels were measured from a drop of blood (5 µl) that was collected from the tail. Blood glucose levels were measured before drug administration, before glucose challenge, and at various times after glucose challenge.

Blood glucose levels were measured by using Accu-Chek Aviva Glucometers (Roche Diagnostics), and levels were reported as milligrams/deciliters. Glucometers were calibrated before each study. Blood (5 µl) was acquired from a tail snip and directly applied to a glucose test strip. Blood levels of MLR-1023 were measured by liquid chromatography/tandem mass spectrometry, and levels were determined by comparing them with a standard curve of MLR-1023 prepared in blood.

Serum Hormone Measurements. Serum was prepared from blood that was collected by retro-orbital eye bleed. Insulin levels were measured from mouse serum by using an Ultra Sensitive Mouse/Rat Insulin ELISA Kit (Crystal Chem Inc.) according to the manufacturer’s directions.

In Vitro and Cell-Based Measurements. Studies of the effects of MLR-1023 on adipocyte differentiation, PPAR (α, δ, and γ) activation, DPP-IV activity, adiponectin production, insulin production, α-glucosidase inhibition, and GLP-1 receptor activation were conducted at ChemBiotek (Kolkata, India). All cell-based systems were validated with a reference compound (described for each assay below). The concentration of MLR-1023 was 10 µM for all assays. Reference compound concentrations are described in Table 1.

In brief, adipocyte differentiation was assessed in mouse 3T3-L1 cells after 8 days of incubation with MLR-1023 or rosiglitazone (10 µM) (Tamori et al., 2002; Li et al., 2007). Adiponectin production was measured in mouse 3T3-L1 cells after 6 days of incubation with MLR-1023 or rosiglitazone (10 µM) (Xu et al., 2004). PPAR (α, δ, and γ) transactivation studies were conducted in transiently transfected COS7 cells containing the appropriate DNA constructs (pGAL4/H9251, pGAL4/H9254, and pGAL4/H9253) coexpressed with transfected cells for 24 h. Luciferase activity was monitored as a measure of PPARα, δ, and γ activation (Staels et al., 1998; Gervois et al., 1999; Raspé et al., 1999; Carmona et al., 2007).

DPP-IV activity was assessed from protease derived from porcine kidney. MLR-1023 or 3-N-[(2S,3S)-2-amino-3-methylpentanoyl]-1,3-thiazolidine (P32/9810 µM) was incubated in the presence of substrate (Gly-Pro-7-amido-4-methylcumarain) for 30 min. Formation of the fluorescent proteolytic cleavage product was monitored as a measurement of DPP-IV activity (Kieffer et al., 1995). Insulin release was assessed in RINm5F cells. MLR-1023 or GLP-1 (5 µM) was incubated with these cells for 60 min, and insulin levels were measured by ELISA (Jehee et al., 1995). α-Glucosidase activity was assessed from enzymes derived from Bacillus subtilis. This activity was measured by monitoring the cleavage of a p-nitrophenyl α-D-glucoside after 60-min incubation with MLR-1023 or castanospermin (Ridrujo et al., 1989). GLP-1 receptor activation was monitored by measuring cAMP production in RINm5F cells after incubation with MLR-1023 or GLP-1 (5 µM) for 30 min (Watanabe et al., 1994).
Kinase Assays. MLR-1023 was evaluated for activity against 47 separate kinases in a Caliper Lifesciences (Hopkinton, MA) kinase-profiling panel. The assay conditions for each kinase examined are described at the Caliper Lifesciences website (www.caliper.com). In brief, for each kinase assay, MLR-1023 (10 μM) was preincubated with kinase and fluoroscein-labeled protein substrate. The reaction was initiated with the addition of ATP (at a concentration at or below the K_m for each kinase), and the level of fluoroscein phosphopeptide was measured. The assays were conducted in duplicate.

Subsequent Lyn kinase assays were conducted at Upstate Ltd (Dundee, Scotland). In brief, MLR-1023 at concentrations ranging from 1 nM to 100 μM was preincubated with Lyn kinase (Upstate Ltd; recombinant human Lyn A; final concentration: 2 μg/ml), reaction buffer (50 mM Tris-HCl, pH 7.5, 0.1 mM EGTA, 0.1 mM sodium orthovanadate, and 0.1% 2-mercaptoethanol), and substrate Poly (Glu4-Tyr) at 4°C. After the preincubation step, the mixture was diluted 1:200 in reaction buffer that contained [32P]ATP (final concentration at 10 μM or as indicated) and incubated for an additional 30 min at 30°C or as otherwise indicated. The amount of 32P-labeled substrate was used as a measure of Lyn kinase activity.

Data Analysis. All data were analyzed by one- or two-way analysis of variance. A post hoc Dunnett’s or Bonferroni test was used to determine statistical differences between treatment groups. Data are expressed as the mean ± S.E.M., unless otherwise indicated. Group sizes ranged from six to eight mice.

Results

Effects of MLR-1023 Administration on Blood Glucose Levels in Euglycemic Mice and in an Oral Glucose Tolerance Test. To investigate whether MLR-1023 modulates blood glucose lowering by increasing insulin levels the compound was administered to normal, freely fed mice. Freely fed mice (vehicle-treated) exhibited blood glucose levels ranging from 138 (baseline) to 177 mg/dl. Baseline insulin levels were 2.3 ng/ml. Administration of MLR-1023 (30 mg/kg i.p.) significantly (p < 0.05) lowered blood glucose levels to 148 and 158 mg/dl, 30 and 90 min after administration, respectively (Fig. 2A). Insulin levels were not significantly affected by MLR-1023 administration (Fig. 2B).

In the OGGT study, glucose administration to fasted vehicle-treated mice evoked a 4-fold increase in blood glucose levels (56–227 mg/dl) with a peak increase observed 30 min after glucose challenge (Fig. 2C). A single administration of MLR-1023 reduced blood glucose levels by 41% to 158 mg/dl. In this study, insulin levels did not increase but trends downward with MLR-1023 administration (Fig. 2D).

Under these dosing conditions (30 mg/kg i.p.) MLR-1023 administration produced a C_max level of 72.8 μM with a T_max of 0.25 h (Fig. 2E). Protein binding of MLR-1023 in mouse serum has been determined to be 99.0% (data not shown).

Therefore, the C_max free plasma concentration of MLR-1023 can be estimated to be approximately 728 nM.

Effects of MLR-1023 on Targets and Mechanisms Associated with Known Diabetes Therapeutics. In an effort to identify mechanisms that may be associated with MLR-1023-mediated blood glucose lowering, MLR-1023 was evaluated in a battery of in vitro and cell-based assays that represented mechanisms associated with T2D drugs and metabolic disease (Table 1). In this broad evaluation, MLR-1023 did not directly affect PPAR γ, α, or δ activation. Moreover, MLR-1023 did not affect DPP-IV activity, GLP-1 receptor function, or α-glucosidase activity. Consistent with in vivo findings (Fig. 2), MLR-1023 administration did not increase insulin secretion in RINm5F cells. MLR-1023 significantly increased adipocyte differentiation and adiponectin production by 3.7- and 19-fold, respectively. These increases, however, were far less than those elicited by rosiglitazone, which increased these parameters by 21- and 140-fold, respectively.

Evaluation of MLR-1023 in an In Vitro Kinase Panel. MLR-1023, at a concentration of 10 μM, was evaluated for activity against 47 different kinases in a cell-free in vitro screen. Incubation of MLR-1023 with Lyn kinase elicited a repeatable 50% increase in enzyme activity. MLR-1023 did not significantly or meaningfully affect any of the other 47 kinases in the screen (Fig. 3).

In studies designed to understand MLR-1023-mediated Lyn kinase activation it was found that the degree of activation depended on a preincubation step before initiation of the reaction with the addition of ATP. MLR-1023-mediated activation of Lyn kinase increased in proportion to the length of preincubation period in the absence of ATP (Fig. 4A). A 2.7-fold increase in Lyn kinase activation was achieved with a 30-min preincubation. Thus, in all subsequent studies, MLR-1023 was preincubated for 30 min with Lyn kinase in the absence of ATP before the start of the kinase reaction.

MLR-1023 elicited a concentration-dependent increase in Lyn kinase activation with a 2.3- and 2.1-fold increase achieved at concentrations of 3 and 10 μM, respectively (Fig. 4B). The data were analyzed by nonlinear regression analysis, and Prism (version 5; GraphPad Software Inc., San Diego, CA) was used to determine an EC50 value. Using the log (agonist) versus response curve-fitting analysis and constraining the lower value to 100 (because this represents the control value) an EC50 of 63 nM was generated. A similar concentration response curve was generated by using concentrations ranging from 1 to 100 μM and under identical conditions. In that study, the effect at 100 μM did not differ from that of 3 or 10 μM (data not shown).
MLR-1023 Mediated Lyn Kinase Activation: ATP Kinetics. MLR-1023-mediated Lyn kinase activation was studied with increasing concentrations of ATP (range 6.25–800 μM). In control conditions (in the absence of MLR-1023), the rate of Lyn kinase activity increased with increasing ATP concentrations from 132 to 881 U/mg. Inclusion of MLR-1023 (100 μM) increased Lyn kinase activity by 3-fold at each ATP concentration tested (Vmax = 1100 U/mg). MLR-1023 did not affect the Km for ATP (control = 22.4 μM; MLR-1023 = 22.6 μM). These data are shown in Fig. 5.

Effects of MLR-1023 in Lyn Kinase Knockout Mice. To further test the hypothesis that the activation of Lyn kinase was responsible for the glucose-lowering activity of MLR-1023, Lyn knockout mice were evaluated for blood glucose levels after fasting, after an oral glucose challenge, and after administration of MLR-1023 and metformin (Fig. 6). In these studies, wild-type and Lyn knockout mice that were fasted for 18 h exhibited equivalent blood glucose levels (45.1 ± 4.2 compared with 47.4 ± 2.7 mg/dl, respectively). Likewise, oral glucose challenge produced an equivalent 4.5-fold increase in glucose levels (peak 224.2 ± 5.5 compared with 207.5 ± 8.6 mg/dl, respectively).

Acute administration of MLR-1023 (30 mg/kg) to wild-type mice treated with MLR-1023 showed a significant 35% reduction (MLR-1023 = 147.4 ± 11.8; vehicle = 224.2 ± 5.5) in peak glucose on an OGTT compared with vehicle-treated mice (p < 0.05) (Fig. 6A). In contrast, MLR-1023-treated Lyn knockout mice exhibited equivalent peak blood glucose levels...
(201.2 ± 13.7 mg/dl) to those of vehicle-treated Lyn knockout mice (207.5 ± 8.7 mg/dl) (Fig. 6B). In a separate study, administration of the gluconeogenesis inhibitor metformin to Lyn knockout mice produced an equivalent 55% blood glucose lowering compared with vehicle in both Lyn knockout mice and wild-type mice (Fig. 6, C and D). The pharmacokinetic profiles of MLR-1023 in knockout and wild-type mice were equivalent (data not shown).

Discussion

Defects in insulin receptor sensitivity and signaling are considered to be critical factors in the pathology of T2D. Restoration of insulin receptor sensitivity, mimicking insulin signaling, or potentiating insulin receptor activity are considered to be relevant pharmacological approaches in the design of next-generation therapies for T2D (Krentz and Bailey, 2005; DeFronzo, 2010). The current studies show that MLR-1023 does not interact with any known type II diabetic therapeutic target, elicits a blood glucose lowering response in vivo in a Lyn-dependent fashion, and selectively activates Lyn kinase in vitro. Ochman et al., 2012 show that MLR-1023 functions as an insulin-sensitizing agent. These results taken together link MLR-1023-mediated Lyn kinase activation to increased insulin sensitivity and ultimately glycemic control.

In freely fed and fasted mice subjected to an OGTT, MLR-1023 administration significantly reduced blood glucose levels. In both instances, serum insulin levels were unaffected. These data indicate that the effects of MLR-1023 were independent of insulin secretion. Other antidiabetic mechanisms were excluded as possible contributors to MLR-1023 activity. In cell-based and in vitro tests, MLR-1023 did not affect PPAR α, δ, or γ activation or insulin secretion, inhibit DPP-IV enzyme activity, or bind to the GLP-1 receptor. It is noteworthy that MLR-1023 increased adiponectin production and adipocyte differentiation. Although these effects were significantly less than that elicited by rosiglitazone, they may ultimately link to the primary MLR-1023 mechanism.

In vitro, MLR-1023 increased Lyn activation with an EC50 of 63 nM and with maximal activation at a concentration of approximately 1 μM. In vivo, administration of MLR-1023 at the active dose level (30 mg/kg) produced a free compound Cmax (i.e., unbound by protein) of 0.73 μM. This alignment of insulin secretion and insulin signaling was achieved without affecting insulin receptor activity or insulin sensitivity.
between the concentration required to fully activate Lyn kinase in vitro and the concentration achieved in vivo for blood glucose lowering is supportive of a relationship between MRL-1023-mediated Lyn kinase activation and blood glucose lowering.

MRL-1023 did not affect the $K_m$ of ATP for Lyn kinase in the activation assay, indicating that MRL-1023 did not interact with the ATP binding site to either mimic or facilitate ATP binding with Lyn kinase. The lack of effect on the ATP $K_m$ suggests that MRL-1023 increased Lyn kinase activity through regulation at an allosteric binding site. Nearly all small-molecule kinase activators act through binding to an allosteric site (Simpson et al., 2009; Eglen and Reisine, 2011). For example, the small-molecule AMP kinase activator, parmitoyltransferase 1, increases activity of the enzyme inhibiting the autoinhibitory phosphorylation state of the enzyme (Pang et al., 2008). Another AMP kinase activator, 6,7-dihydro-4-hydroxy-3-(2'-hydroxy[1,1'-biphenyl]-4-yl)-6-oxo-thieno[2,3-b]pyridine-5-carbonitrile (A-769,662), acts by binding to an allosteric binding site, which in turn drives kinase activity (Zhang et al., 1999; Salituro et al., 2001).

Lyn kinase activity is tightly controlled by its phosphorylation state (Donella-Deana et al., 1998; Ingleby, 2008). Phosphorylation of Lyn kinase at the Tyr-397 residue within its activation loop results in increased activity (Donella-Deana et al., 1998). In contrast, phosphorylation at the C-terminal tail Tyr-508 residue inhibits activity by inducing its association with an internal Src homology 2 domain (Donella-Deana et al., 1998). Lyn kinase is autophosphorylated at both sites (Ingleby, 2008). In addition, Lyn kinase can exist in a nonphosphorylated, partially active state (Ingleby, 2008). However, Lyn kinase is unlikely to exist in the nonphosphorylated form because of its propensity to autophosphorylate. Thus, it is unlikely that MRL-1023 induces a conformational change to the enzyme that shifts it to a fully activated nonphosphorylated form.

The existing data surrounding small-molecule activators of kinases and data showing that Lyn is tightly regulated by phosphorylation state suggest several possible mechanisms by which MRL-1023 could modulate Lyn activation. MRL-1023 may affect one of the two known phosphorylation regulatory sites on Lyn. For example, MRL-1023 may modify the kinase to make it more amenable to phosphorylation at the activation site (Tyr-397) or inhibit phosphorylation at the inhibition site (Tyr-508). Alternatively, MRL-1023 could bind at the regulatory loop associated with Tyr-397 so as to stabilize the loop in its activating configuration independent of Tyr-397 phosphorylation state. Finally, MRL-1023 could affect kinase activity independent of modifying the phosphorylation state by, for example, inhibiting Src homology 2 site interaction with the phosphorylated Tyr-508 of Lyn and relieving the autoinhibitory effects of this interaction.

The most compelling data linking MRL-1023-mediated Lyn kinase activation to blood glucose-lowering effects in vivo are provided by the results in Lyn kinase knockout mice. In these studies, MRL-1023 did not lower blood glucose levels in Lyn kinase knockout mice subjected to an OGTT. These data show that MRL-1023-mediated blood glucose lowering depends on the presence of Lyn kinase. Although these data do not necessarily indicate that MRL-1023 directly activates Lyn kinase in vivo, they do show a requirement of Lyn kinase for MRL-1023-mediated blood glucose lowering. It is noteworthy that fasted blood glucose levels and peak glucose levels were equivalent in both wild-type and Lyn knockout mice, suggesting the presence of Lyn is not a requirement for
maintaining glucose homeostasis. Furthermore, metformin lowered glucose after a glucose challenge in both wild-type and knockout animals, showing that MLR-1023 and metformin administration affect glucose levels by different mechanisms and these mice are responsive to some forms of pharmacologically mediated blood glucose lowering.

The known involvement of Lyn kinase in insulin signaling and the data from the current studies indicate that MLR-1023-mediates blood glucose lowering through a Lyn kinase-dependent mechanism. Src-related kinases, and especially Lyn kinase, are directly involved in insulin signaling (Lebrun et al., 1998; Müller et al., 2001a,b, 2005). In adipocytes, activated Lyn kinase directly promotes the phosphorylation of IRS-1 and in turn promotes insulin signaling including glucose transporter type 4 translocation (Müller et al., 2001b, 2005). However, Ochman et al. (2012) clearly showed that MLR-1023 does not function as an insulin mimetic but as a potentiator of insulin activity. It seems that MLR-1023-mediated Lyn kinase activation and IRS-1 phosphorylation, in the absence of insulin, are not sufficient to promote glucose utilization in vivo. There are multiple phosphorylation sites on IRS-1, and it may be that these phosphorylation sites are act on by Lyn overlap or are distinct from those sites phosphorylated by the insulin receptor. Thus, the coordinated phosphorylation of IRS-1 by Lyn and the insulin receptor may induce a more active IRS-1 form that manifests as a potentiated insulin-mediated blood glucose-lowering response in vivo.

Glimepiride is a well described insulin secretagogue that also promotes blood glucose-lowering activity independent of its action on insulin secretion (Müller, 2000). It is noteworthy that this activity of glimepiride has also been related to the activation of Lyn kinase (Müller et al., 2001a, 2005). However, unlike MLR-1023, this Lyn kinase activation is nonselective and indirectly regulated through effects on membrane-associated lipid rafts (Müller et al., 2005). Nevertheless, these data support the concept that activation of Lyn kinase is a viable mechanism for regulating blood glucose in patients with T2D.

The data generated from the current studies and the results shown in Ochman et al. (2012), coupled with the known involvement of Lyn kinase in insulin-mediated signaling, support a model where MLR-1023 promotes insulin signaling through the activation of Lyn kinase. The data from these studies show that MLR-1023 activates Lyn kinase in vitro and its blood glucose-lowering effects depend on the presence of Lyn kinase in vivo. The results from both articles describe MLR-1023 as a mechanistically novel, putative therapeutic for the treatment of T2D.

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Participated in research design: Saporito, Ochman, Lipinski, Handler, and Reaume.
Conducted experiments: Saporito and Ochman.
Contributed new reagents or analytic tools: Lipinski.
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