Design and Synthesis of Novel Pyridoxine 5′-Phosphonates as Potential Antiischemic Agents

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On the basis of previous reports that the natural cofactor pyridoxal 5′-phosphate **1** appears to display cardioprotective properties, a series of novel mimetics of this cofactor were envisioned. As pyridoxal 5′-phosphate is a natural compound and is subject to biological degradation and elimination pathways, the objective was to generate active phosphonates that are potentially less light sensitive and more stable in vivo than the parent vitamer. Several phosphonates were designed and synthesized, and in particular, compounds **10** and **14** displayed similar biological traits to natural phosphate **1** in the rat model of regional myocardial ischemia and reperfusion. A reduction in infarct size was observed in animals treated with these compounds. In an effort to identify other relevant cardioprotective models in order to potentially define structure−activity relationships, these three compounds were tested in the rat working heart model. Compounds **1**, **10**, and **14** were compared to dichloroacetic acid (DCA) as positive control in this model. As with DCA, compounds **1**, **10**, and **14** were found to induce a shift from fatty acid oxidation toward glucose oxidation.

Introduction

In 1949, Rinehart and Greenberg observed that monkeys given a vitamin B6 deficient diet developed atherosclerosis.[1] More recent studies using isolated perfused rat hearts have suggested that pyridoxal 5′-phosphate (PLP) is an effective ATP receptor antagonist with activity in the 10−50 μM range.[2] In humans, the situation is more complex, and work on optimizing the therapeutic benefits of vitamin B6 in treating heart disease is ongoing. Reports have confirmed the fact that patients who have suffered a myocardial infarction display lower levels of PLP.[3] Suggestive evidence has been provided to support the notion that a high daily dose of supplemental pyridoxine (PN) may delay or even prevent cardiovascular events.[4] The authors of this study postulated that the protective effect of vitamin B6 was attributed to increased levels of PLP, which is the active coenzyme form required for the catabolism of homocysteine. Elevated levels of this atherogenic amino acid are now considered to be an independent risk factor for atherosclerotic disease,[5] and the clinical relevance of the inverse relationship between homocysteine and B vitamin levels is still under investigation.[6,7] It has been further postulated that pyridoxine supplementation can significantly improve endothelial function, and potential beneficial effects in terms of the reduction of transplant coronary artery disease have been noted.[8]

The vitamin B6 group primarily consists of three interrelated vitamers including pyridoxine, pyridoxal (PL) and pyridoxamine (PM). All three pyrimidine derivatives are found in tissue as their corresponding 5′-phosphates. In particular, **1** (Figure 1), the metabolically active coenzyme form of vitamin B6, plays a vital role as a cofactor in several crucial enzymatic pathways and is formed by the in vivo oxidation of the corresponding pyridoxine 5′-phosphate **2**. The major degradation pathway for PLP in vivo is its conversion to pyridoxal by the action of alkaline phosphatase. There is sufficient evidence to warrant further exploration into the possibility that the B6 vitamins may serve as potential leads for the design of agents for the treatment of certain cardiovascular diseases. Such agents should be designed to selectively mimic one or more of the known modes of actions of the vitamin B6 cogeners with similar potency as these natural compounds. Along this line, mimetics functionally similar to PLP have been reported.[9]

The phosphate moiety is a common feature in several important biomolecules. In particular, this epitope serves to target well-defined phosphate binding pockets of a variety of enzymes. It is therefore of interest to develop novel phosphate mimetics of natural compounds that can potentially target phosphate binding pockets, but are themselves relatively resistant to degradation by phosphatase enzymes. The replacement of a carbon atom for the oxygen in the P−O−C phosphate bond renders the resulting phosphonate linkage relatively resistant toward chemical and enzymatic hydrolysis. Additionally, the phosphonate substitution pattern PCR^1R^2C allows for a wide range of structural modifications including variations in both substituents R^1 and R^2 at the tetravalent carbon center. For example, Blackburn has pioneered the use of α-fluorophosponates (R^1 = F and R^2 = H) and difluorophosphonates (R^1 and R^2 = F) as phosphate surrogates.[10] The biological activity of the former class of mimetics, however, remains relatively unexplored.[11] The second deproto-

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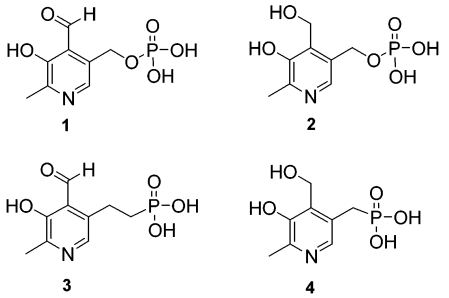


Figure 1. B-Vitamers and phosphonate analogues.

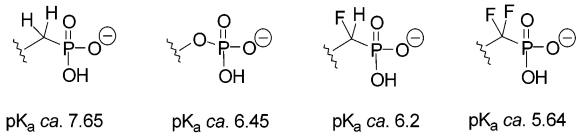
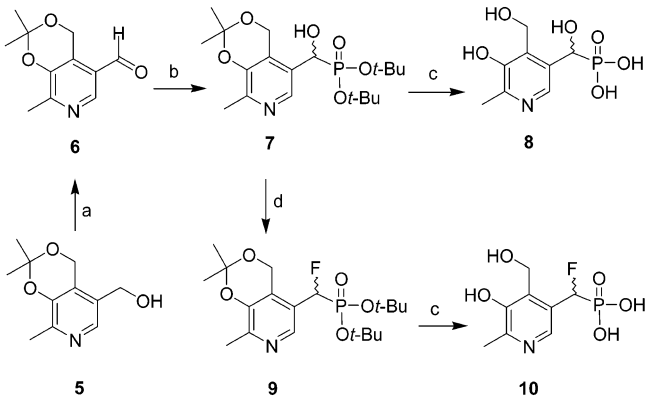


Figure 2. Acidity (pK_a of the second deprotonation) of phosphate/phosphonates.

nation constants for these phosphatase stable mimetics are shown in Figure 2.[12] In protein binding events at physiological pH, it is generally assumed that the natural phosphate moiety is in the completely ionized state. It can therefore be anticipated that the increased acidity for both the CHF- and CF_2-phosphonates would not impair their ability to function as mimetics. In fact, the pK_a of the former is nearly identical to that of the natural phosphonate.

Pyridoxal 5′-phosphate-dependent enzymes are involved in the catalysis of many important metabolic processes. The phosphate moiety typically binds near the N terminus of the anchoring α-helix, and in concert with Schiff base formation with an active-site lysine, determines the placement and conformation of **1**.[13] Recently, there has been renewed interest in the application of phosphonate analogues of **1**. The ethylphosphonate **3**[14] has been utilized as a structural probe in the detailed investigation into the ionization state of **1** in glycogen phosphorylase.[15] Meanwhile, the 4-formyl derivative of phosphonate derivative **4** served as a precursor for the development of pyridoxal-6-arylazo-5′-phosphonate-based P2 receptor antagonists.[16] Additionally, beneficial effects in animal models have indicated the possibility of treating cardiovascular pathologies such as ischemia reperfusion injury with natural cofactor **1**.[17] Ischemia typically occurs in an organ that fails to receive a sufficient supply of blood, and this situation leads to structural and functional abnormalities. Subsequent resumption of blood flow, known as ischemic reperfusion, additionally contributes to injury of the organ. When the organ affected by these insults is the heart, the condition is referred to as myocardial ischemia reperfusion injury. One of the primary goals in the treatment of acute myocardial infarction is the early and total restoration of infarct-related artery perfusion. In the present study, we describe the preparation of a series of pyridoxine 5′-phosphonates as agents that potentially mimic the efficacy of the natural cofactor in reducing scar size in rats that have been subjected to ischemia reperfusion injury.

Scheme 1. Synthesis of Pyridoxine 5′-Phosphonates **8** and **10**[a]



[a] Reagents and conditions: (a) MnO_2, C_7H_8, 40 °C; (b) (*t*-BuO)$_2$PO$^-$Na$^+$, THF, 0 °C → rt; (c) HOAc:H_2O 4:1, 75 °C; (d) DAST, CH_2Cl_2, −78 °C → rt.

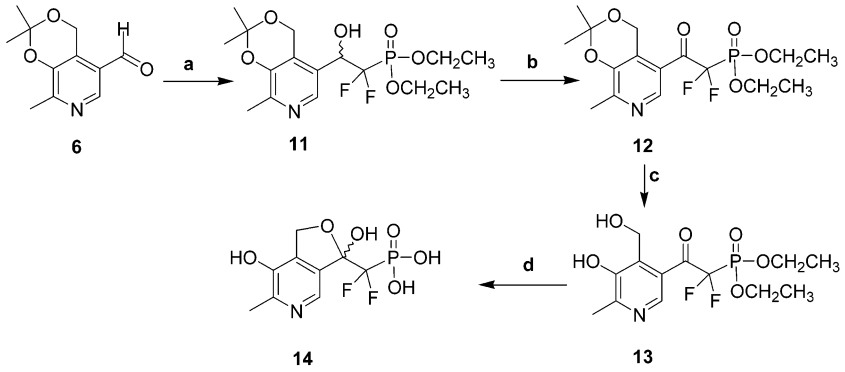
Results and Discussion

Chemistry. The synthesis of pyridoxine 5′-phosphonates **8** and **10** is shown in Scheme 1. The key starting material for the preparation of both of these phosphonates was the aldehyde **6**, which was in turn obtained from its precursor 5′-alcohol following the procedure of Korytnyk and Ikawa,[18] with slight modification. Thus, mild oxidation of a toluene solution of **5**[19] with manganese(IV) oxide at 40 °C gave pyridoxal derivative **6** in 83% yield. Formation of blocked phosphonate **7** was accomplished by the addition of **6** to a solution of di-*tert*-butyl phosphite anion in THF at 0 °C,[20] followed by reaction at room temperature for 1 h. Aqueous workup and subsequent recrystallization gave the racemic α-hydroxyphosphonate **7** in 82% yield. Concomitant removal of all protecting groups was achieved by heating a solution of blocked **7** in acetic acid: water 4:1 at 75 °C overnight. The deprotected α-hydroxyphosphonate **8** precipitated from the solution upon cooling to room temperature and, combined with a second crop, was obtained in 89% yield.

We next focused our attention on the substitution of a single hydrogen for fluorine in phosphonate template **4**. The preparation of **10** was initiated from **7**. Thus, direct substitution of the hydroxyl group by fluorine upon treatment with DAST (1.3 equiv) proceeded smoothly to provide the **9** in 82% yield. Routine deprotection as before, gave the target α-fluorophosphonate **10** in good yield.

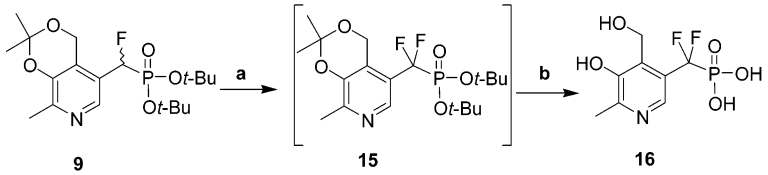
The synthesis of α,α-difluoroketophosphonate **14** is outlined in Scheme 2. Thus, treatment of blocked aldehyde **6** with the lithium salt of diethyl difluoromethylphosphonate (1.0 equiv) in THF provided difluorohydroxyphosphonate **11** in excellent (80%) yield. Subsequent oxidation with manganese(IV) oxide at 60 °C in toluene overnight furnished **12** (48% yield). The pyridoxyl ring H-6 proton of this diketophosphonate was significantly downfield shifted to 8.87 ppm as anticipated. Two-step deprotection involving removal of the isopropylidine protection under acidic conditions to provide **13**, followed by treatment with triethylsilyl bromide in acetonitrile, provided phosphonic acid **14**. The absence of a ^{13}C carbonyl carbon resonance (190−

Scheme 2. Synthesis of Difluoroketophosphonate **14**[a]



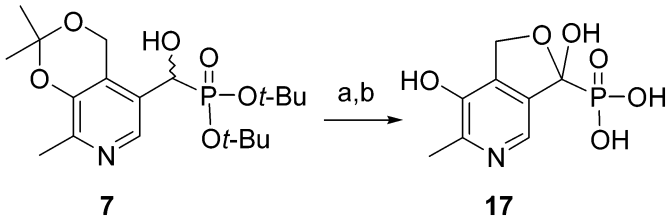
[a] Reagents and conditions: (a) LDA/*n*-BuLi/$CHF_2PO(OCH_2CH_3)_2$, THF, −78 °C → rt; (b) MnO_2, C_7H_8, 60 °C; 18 h; (c) HOAc:H_2O 4:1, 80 °C, 5 h; (d) $(CH_3CH_2)_3SiBr/CH_3CN$.

Scheme 3. Synthesis of Difluorophosphonate **16**[a]



[a] Reagents and conditions: (a) LDA/THF, $(C_6H_5SO_2)_2NF$, −78 °C; (b) HOAc:H_2O 4:1, 75 °C, 18 h.

Scheme 4. Synthesis of Phosphonate **17**[a]



[a] Reagents and conditions: (a) $[(CH_3)_2CH]_2NCH_2CH_3$ /CH_2Cl_2, SO_3−Py/DMSO, −8 °C, 1.5 h; (b) HOAc:H_2O 4:1, 75 °C, 18 h.

200 ppm) suggested that this compound, and possibly precursor **13**, were in their respective cyclic hemiketal forms.

The synthesis of difluorophosphonate **16** is shown in Scheme 3. Treatment of a solution of the lithium anion of **9** in THF at −78 °C with excess NFSI (1.33 equiv) gave crude **15** after workup. The crude blocked difluorophosphonate was unstable and was immediately deprotected without prior purification. Purification of the final deprotected material by silica gel chromatography gave **16**, albeit in modest yield (38%). Scale-up preparation of this compound was difficult, and sufficient amounts of **16** of consistent purity for subsequent biological evaluation proved to be elusive. The synthesis of phosphonate **17** (Scheme 4), however, was straightforward; thus, oxidation of the α-hydroxyl moiety, followed by immediate deprotection after workup, gave cyclic hemiketal **17** in good yield.

Ischemia Reperfusion Studies. Ischemia-reperfusion injury is a root cause of a majority of the important cardiovascular diseases including myocardial infarction and thrombotic stroke. Myocardial ischemia is a condition that exists when the uptake of oxygen in the heart is below the level needed to maintain the rate of cellular oxidation.[21] Prolonged myocardial ischemia leads to significant tissue injury and ultimately myocyte necrosis. Efforts to establish the precise sequence of biochemical events involved in recovery upon reperfusion or cell death are ongoing. A detailed understanding of these processes would hopefully pave the way for the rational development of treatment regimens to delay myocardial cell death. Nevertheless, myocardial infarct size is well established as an important predictor of cardiac function and thus prognosis.[22]

We investigated the ability of our compounds to reduce infarct size in the male Wistar rat model of left anterior descending coronary artery occlusion (25 min) and reperfusion (2 h).[23] A comparison of compounds **1**, **8**, **10**, and **14** (bolus injection of 0.02 mmol/kg) for their ability to reduce infarct size relative to saline control is shown in Figure 3. The reduction in infarct size under these conditions was found to be 33% (SEM ± 3, $p < 0.05$), 19% (SEM ± 7, $p > 0.05$), 35% (SEM ± 3, $p < 0.05$), and 46% (SEM ± 2, $p < 0.05$), respectively. As the reduction of infarct size with 0.02 mmol/kg of compound **8** was not found to be statistically significant ($p > 0.05$), we reevaluated this compound along with pyridoxine phosphate **2** and literature compounds **3** and **4** in the rat model of ischemia reperfusion injury at a higher dose of 0.1 mmol/kg ($n = 10$ for each group). None of these compounds were found to significantly reduce infarct size; however, a trend toward a reduction in infarct size (20%, SEM ± 5, $p = 0.07$) was noted with compound **4**. The reduction in infarct size with compound **8** (0.1 mmol) as the treatment again did not reach statistical significance.

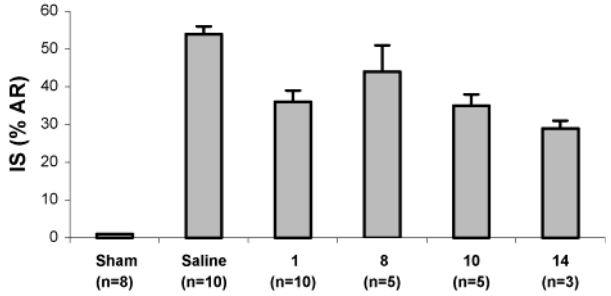


Figure 3. Effects of compounds **1**, **8**, **10**, and **14** on infarct size after ischemia (25 min) followed by reperfusion (120 min). The compounds (0.02 mmol/kg) were administered via bolus injection 5 min prior to occlusion. Infarct size was determined by staining the heart with *p*-nitro-blue tetrazolium (NBT, 0.5 mg/mL) for 40 min at 37 °C. The infarct size (IS) is reported as a percentage of the area at risk (AR) (standard error is indicated by the error bars).

TCA Cycle Studies. As glycogen phosphorylase requires PLP as a cofactor, we decided to screen select phosphonates for beneficial metabolic modulation properties. Under normal physiological conditions, the heart primarily utilizes carbohydrates and fatty acids as convenient energy sources in order to maintain cardiac homeostasis. Under certain pathological conditions where plasma fatty acid levels become elevated, such as diabetes mellitus, or during myocardial infarction, alterations in energy metabolism occur. In these pathologies, there is a dramatic reduction in glucose oxidation with fatty acid oxidation predominating.[24] This increased fatty acid oxidation has recently been linked to increased ischemic injury that is due, in part, to a significant uncoupling between glycolysis and glucose oxidation during reperfusion. The resulting imbalance subsequently leads to increased lactate release along with increased proton generation from glycolytically derived ATP, which in turn leads to greater Na^+ and thus Ca^{2+} influx.

Recently, a novel therapeutic approach for the treatment of ischemic heart disease has been proposed based on the concept of modulating myocardial metabolism.[25] The approach essentially targets increasing glucose oxidation at the expense of fatty acid oxidation. The net effect of this shift in metabolism is the improvement of cardiac efficiency due to better oxygen utilization along with decreased acidosis in the myocardium resulting from improved coupling of glycolysis to glucose oxidation.

One such metabolic modulator, namely dichloroacetic acid (DCA), has recently been shown to behave as a direct pyruvate dehydrogenase complex activator (PDH activator).[26] The PDH complex irreversibly catalyzes oxidation of pyruvate to acetyl CoA, and this carbohydrate-derived acetyl CoA can then directly participate in the tricarboxylic acid cycle in mitochondria. The resultant elevation in the level of glucose oxidation at the expense of fatty acid oxidation can potentially improve the mechanical function of the reperfused human heart. We therefore investigated the effect of compound **1** and two phosphonates, namely **10** and **14**, for their ability to alter cardiac metabolism.

The compounds were tested in the Langendorff perfusion model following published protocol.[27] Working rat hearts were perfused with modified Krebs−Henseleit solution containing calcium (2.5 mmol/L), glucose (5.5 mmol/L), palmitate (0.4 mmol/L), and 3% bovine serum albumin. Fatty acid oxidation and glucose oxidation were measured simultaneously by perfusing hearts with [^{3}H]-palmitate and [^{14}C]-glucose, respectively. The effect of compounds **1**, **10**, and **14** on the fatty acid utilization rate is shown in Figure 4. DCA (positive control) did not alter palmitate oxidation rates as compared to the untreated control group (397 ± 29 vs 418 ± 24, respectively). Phosphate **1** (398 ± 15) and phosphonates **10** (409 ± 17) and **14** (414 ± 12) likewise failed to alter palmitate oxidation compared to control (418 ± 24).

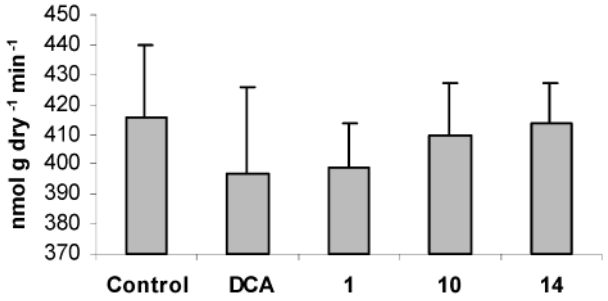


Figure 4. Effects of dichloroacetic acid (positive control) and compounds **1**, **10**, and **14** on metabolic flux through fatty acid oxidation. The hearts were perfused with either TCA (3 mM) or test compound (1 mM), while the control group was untreated ($n = 6$ hearts in each group).

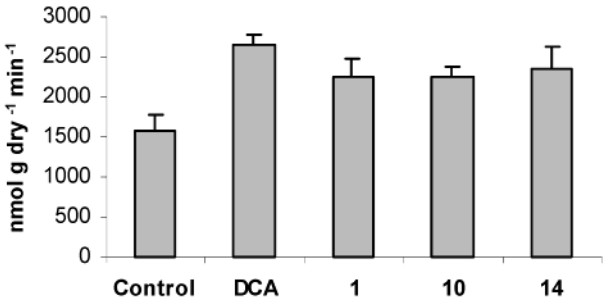


Figure 5. Effects of dichloroacetic acid (positive control) and compounds **1**, **10**, and **14** on metabolic flux through glycolysis. The hearts were perfused with either TCA (3 mM) or test compound (1 mM), while the control group was untreated ($n = 6$ hearts in each group); $p < 0.05$ vs control for DCA, **1** and **10**, $p = 0.052$ for compound **14**.

Glucose oxidation rates were determined by the qualitative measurement of $^{14}CO_2$ production.[28] As shown in Figure 5, DCA effected an increase in glucose oxidation rates as compared to control {2422 ± 140 ($p = 0.001$) vs 1580 ± 183}, as did each of compounds **1**, **10**, and **14** {2253 ± 230 ($p = 0.045$), 2244 ± 137 ($p = 0.016$), 2339 ± 293 ($p = 0.052$), vs 1580 ± 183}.

As shown in Figure 6, the contribution from palmitate oxidation and glucose oxidation in the control under the conditions employed in this study were nearly identical (%52 ± 2 vs %48 ± 2). In the DCA-treated hearts, the contribution from these substrates was altered significantly. Treatment of rat hearts with this agent resulted in a decrease in contribution from palmitate oxidation with a concomitant increase in glucose oxidation (%37 ± 2 vs %63 ± 2, respectively). Compounds **1**, **10**, and **14** were found to decrease the contribution from palmitate oxidation and simultaneously increase the glucose oxidation contribution (%42 ± 1 vs %58 ± 2; %43 ± 1 vs %57 ± 2; and %42 ± 1 vs %58 ± 2, respectively).

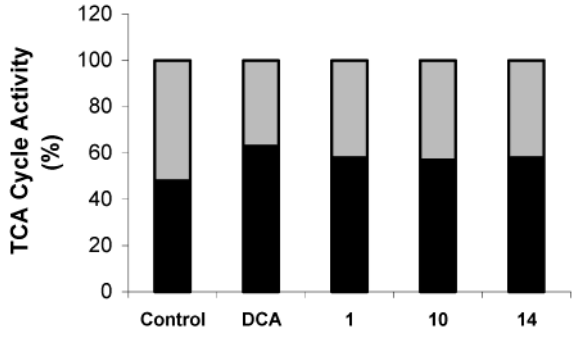


Figure 6. Effects of DCA and compounds **1**, **10**, and **14** on the TCA cycle contribution; % palmitate, light gray shading; % glucose, black shading.

As the phosphonates described herein are based on a natural vitamer scaffold that behaves as a cofactor in numerous enzymatic processes, an acute toxicity test was performed on the monosodium salt of compound **10** (prepared by titrating this compound with 1 equiv of standard sodium hydroxide solution). The LD_{50} from intravenous administration of this salt (12 mg/mL) to rats was found to be greater than 100 mg/kg.

Conclusion

In contrast to aldehydes **1** and **3** that are known to be light sensitive, phosphonates **8**, **10**, **14**, and **17** were found to be stable for 24 h in aqueous solutions kept at room temperature unshielded from light as assayed by HPLC (see Experimental Section). Ischemia reperfusion injury represents an extremely complex pathology. Active B-vitamer **1** and phosphonate derivates **10** and **14** have been shown to reduce infarct size in preliminary experiments in the rat model of ischemia reperfusion injury. At least one general pathway for their potential action in the nonischemic heart has been identified, namely that of involvement in energy metabolism. Neither DCA, nor compounds **1**, **10**, and **14**, displayed any measurable influence on palmitate oxidation rates in the isolated working rat heart. These compounds did, however, alter glucose metabolism. The experimental conditions employed for the metabolic studies were designed to provide equal contributions from fatty acids and glucose to the TCA cycle. DCA and compounds **1**, **10**, and **14** were able to induce a desirable shift away from fatty acid metabolism toward glucose metabolism. Hemodynamic parameters including heart rate, mean arterial pressure, and pressure rate index were measured for all animal groups in the metabolic study. No significant differences in these three parameters were observed between the test animals and control group, demonstrating that the observed effects on glucose metabolism are not occurring secondarily to changes in contractile function. Although these studies were performed on nonischemic hearts, an increase in glucose oxidation both during and postischemia would be expected to provide therapeutic benefit by improving the coupling of glycolysis and glucose oxidation which leads to a decrease in intracellular proton production.[28] The net effect of improved coupling is therefore an improvement of cardiac efficiency. Compounds **1**, **10**, and **14** were able to induce a shift toward glucose oxidation, and further work to uncover more details on their specific mode of action is currently underway.

Experimental Section

Pyridoxine was purchased from Fluka. All other reagents were obtained from standard commercial sources and were used without further purification unless otherwise indicated. All reactions were carried out under a dry nitrogen atmosphere using dry solvents. Reactions were monitored by thin-layer chromatography carried out on Sigma-Aldrich 0.25 mm silica gel plates (60 Å, fluorescent indicator). Spots were visualized under UV light (254 nm). Flash column chromatography was performed on silica gel (60, particle size 0.032−0.063 mm) from Scientific Adsorbents Inc. Reverse phase chromatography was performed on C-18 silica gel (60, particle size 0.032−0.063 mm) from Scientific Adsorbents Inc. HPLC analysis was performed using a Waters 996 PDA High-Performance Liquid Chromatograph equipped with a Waters 600 Controller using a gradient elution; 0−100% (v/v) methanol or acetonitrile vs 0.1% aqueous trifluoroacetic acid over 20 min; 1 mL/min flow rate @ 25 °C; Zorbax SB−C8 (4.6 × 150 mm; 3.5 μm particle size) column equipped with a Zorbax SB-Aq (4.6 × 12.5 mm; 5 μm particle size) guard column. Signals were detected with a photodiode array detector (set at maxplot 254−400 nm). NMR spectra were recorded on a Bruker AM-300 instrument (^{13}C, ^{19}F, and ^{31}P at 75.5, 282, and 121 MHz, respectively) and were calibrated using residual nondeuterated solvent as the internal reference. Spectra were processed using Spinworks version 1.3 (developed by Dr. Kirk Marat, Department of Chemistry, University of Manitoba). All ^{19}F spectra are reported using hexafluorobenzene (δ −162.9 ppm) as the external standard, while ^{31}P spectra were collected using 85% H_3PO_4 (δ 0.0 ppm) as the external reference. Compound **3** was prepared according to the method of Hullar,[14] and compound **4** was prepared following the procedure published by Kim et al.[16]

2,2,8-Trimethyl-4*H*-[1,3]dioxino[4,5-*c*]pyridine-5-carboxaldehyde (6). The reaction conditions employed were similar to those previously reported[18] with minor modification. To a solution of **5**[17] (25 g, 0.12 mol) in toluene (900 mL) was added manganese(IV) oxide (85%), 33 g, 0.32 mol), and the reaction was stirred under inert atmosphere at 40 °C for 4 h. After this time, a second portion of MnO_2 (16.5 g, 0.16 mol) was added, and stirring was continued for an additional 4 h. The solvents were evaporated, and the resulting yellow solid was dissolved in hexane:diethyl ether 1:1 and filtered and the filtrate kept at rt. The resulting solid was collected by filtration, and the mother liquors were recrystallized from hexane:diethyl ether 7:3 to give 20.6 g (83% combined yield) of **6**. This material was of sufficient purity for use in the subsequent reaction.

[Hydroxy-(2,2,8-trimethyl-4*H*-[1,3]dioxino[4,5-*c*]pyridin-5-yl)methyl]phosphonic Acid Di-*tert*-butyl Ester (7). Di-*tert*-butyl phosphite (44.1 g, 227 mmol) was added to a suspended solution of sodium hydride (9.1 g, 60% in mineral oil, 227 mmol) in THF (100 mL) under nitrogen atmosphere at 0 °C. The mixture was warmed to rt and stirred for 15 min. The reaction mixture was again cooled to 0 °C, and a solution of aldehyde **6** (34.2 g, 165 mmol) in THF (300 mL) was added. The resulting mixture was slowly warmed to rt and then stirred for 1 h further. After this time, the reaction was quenched with saturated aqueous $NaHCO_3$ (100 mL). The organic compounds were extracted with diethyl ether, and the organic solution was successively washed with saturated aqueous $NaHCO_3$ and brine, dried ($MgSO_4$), filtered, and evaporated. The resulting crude solid was dissolved in hexane: diethyl ether 52:48 (500 mL) and kept at rt overnight. The colorless solid was collected by filtration and washed with hexane then diethyl ether (quickly). The mother liquor was evaporated, washed with hexane, and again recrystallized to provide 54.5 g (82% combined yield) of **7** as a colorless solid. ^1H NMR ($CDCl_3$) δ 8.13 (d, J = 2.6 Hz, 1H), 5.12 (dd, J = 16.4, 1.9 Hz, 1H), 4.92 (dd, J = 16.4, 1.4 Hz, 1H), 4.73 (dd, J = 10.6, 3.6 Hz, 1H), 3.00 (dd, J = 10.6, 3.9 Hz, 1H), 2.40 (d, J = 2.0 Hz, 3H), 1.56 (s, 3H), 1.52 (s, 3H), 1.47 (s, 9H), 1.43 (s, 9H). ^{31}P NMR ($CDCl_3$, ^1H-decoupled) δ 13.3. HRMS (ES^+) calcd for $C_{19}H_{33}NO_6P$ ($M + H^+$) m/z, 402.204; found 402.2039. Anal. ($C_{19}H_{32}NO_6P$): C, H, N.

[Hydroxy-(5-hydroxy-4-hydroxymethyl-6-methylpyridin-3-yl)methyl]phosphonic Acid (8). The fully protected compound **7** (48.9 g, 120.5 mmol) was stirred in acetic acid−water (4:1, 800 mL) at 75 °C for 17 h. After this time, the reaction mixture was allowed to cool to rt, and the precipitate was collected by filtration. The colorless solid was washed with water, methanol, and then ethyl acetate. The mother liquor was evaporated and washed with water, methanol, and ethyl acetate. The HPLC profile for both solids was found to be identical, thus furnishing 26.8 g (89% combined yield) of **8** as a colorless solid. ^{1}H NMR (D_2O) δ 8.24 (d, J = 2.2 Hz, 1H), 5.25 (d, J = 13.5 Hz, 1H), 5.05 (d, J = 14.0 Hz, 1H), 4.97 (d, J = 14.0 Hz, 1H), 2.63 (d, 1.2 Hz, 3H). ^{1}H NMR (0.1 M NaOH in D_2O): δ 7.73 (d, J = 2.0 Hz, 1H), 5.09 (d, J = 12.3 Hz, 1H), 4.87 (d, J = 12.3 Hz, 1H), 4.65 (d, J = 12.3 Hz, 1H), 2.41 (d, J = 0.8 Hz, 3H). ^{13}C NMR (0.1 M NaOH in D_2O) δ 161.0, 141.9 (J = 1.4 Hz), 139.5 (d, J = 4.3 Hz), 137.9 (d, J = 1.3 Hz), 124.4 (d, J = 2.9 Hz), 68.2 (d, J = 141.7 Hz), 55.6, 15.6. ^{31}P NMR (D_2O, ^{1}H-decoupled) δ 14.8; (0.1 M NaOH in D_2O) δ 14.3 (d, J = 12.3 Hz); ^{1}H decoupled δ 14.3 (s). HRMS (ES^+) calcd for $C_8H_{13}NO_6P$ ($M + H^+$) m/z, 250.0475; found 250.0477. HPLC 99% purity. Anal. ($C_8H_{12}NO_6P$): C, H, N.

[Fluoro-(2,2,8-trimethyl-4*H*-[1,3]dioxino[4,5-*c*]pyridin-5-yl)methyl]phosphonic Acid Di-*tert*-butyl Ester (9). Diethylaminosulfur trifluoride (1.0 g, 6.2 mmol) was added to a solution of **7** (1.9 g, 4.73 mmol) in dichloromethane (200 mL) at −78 °C under nitrogen atmosphere. The resulting solution was allowed to slowly warm to rt and then stirred for an additional 1.5 h. Saturated aqueous $NaHCO_3$ (100 mL) was added slowly to quench the reaction, After separation of the phases, the aqueous layer was further extracted with dichloromethane (2×). The combined organic solution was dried ($MgSO_4$), filtered, and evaporated. Flash chromatography (silica, hexane:ethyl acetate 9:1 to 1:1) gave 1.56 g (82%) of **9** as yellow oil. ^{1}H NMR **(**$CDCl_3$) δ 8.01 (s, 1H), 5.39 (dd, J = 44.5, 9.1 Hz, 1H), 5.06 (dt, J = 16.9 Hz, J = 1.4 Hz, 1H), 4.99 (d, J = 16.9 Hz, 1H), 2.39 (dd, J = 1.4, 1.4 Hz, 3H), 1.53 (s, 3H), 1.50 (s, 3H), 1.43 (s, 9H), 1.42 (s, 9H). ^{19}F NMR ($CDCl_3$, ^{1}H decoupled) δ −202.87 (d, J = 84.9 Hz). ^{31}P NMR ($CDCl_3$, ^{1}H decoupled) δ 6.86 (d, J = 84.9 Hz). HRMS (ES^+) calcd for $C_{19}H_{32}NO_5FP$ ($M + H^+$) m/z, 404.1997; found 404.1995.

[Fluoro-(5-hydroxy-4-hydroxymethyl-6-methylpyridin-3-yl)methyl]phosphonic Acid (10). Fully protected compound **9** (1.56 g, 3.8 mmol) was stirred in acetic acid:water 4:1 (30 mL) at 75 °C for 15 h. The solvents were removed, and the residue was coevaporated with toluene to give a yellow solid. This crude product was dissolved in hot water (20 mL) and filtered while still hot. The filtrate was slowly cooled to fridge temperature (5 °C), and the precipitated product was collected by filtration. The solid was washed with cold water (10 mL), methanol (10 mL), and dichloromethane (10 mL) to give a colorless solid. The mother liquor was subsequently evaporated, dissolved in water (10 mL), and precipitated repeatedly to provide additional colorless solid. The HPLC profile for both solids was found to be identical, and 484 mg (50% combined yield) of **10** was obtained as a colorless solid. ^{1}H NMR (0.1 M NaOH in D_2O) δ 7.71 (d, J = 1.6 Hz, 1H), 5.87 (dd, J = 44.4, 8.2 Hz, 1H), 4.83 (d, J = 12.6 Hz, 1H), 4.64 (d, J = 12.6 Hz, 1H), 2.43 (s, 3H). ^{13}C NMR (0.1 M NaOH in D_2O) δ 160.4, 143.1, 138.7 (d, J = 3.6 Hz), 134.5 (d, J = 17.8 Hz), 124.4, 89.2 (dd, J = 177.5, 145.5 Hz), 55.68, 15.7. ^{19}F NMR (0.1 M NaOH in D_2O) δ −196.8 (dd, J = 69.0, 44.4 Hz); ^{1}H-decoupled δ −196.8 (d, J = 69.0 Hz). ^{31}P NMR (0.1 M NaOH in D_2O) δ 9.38 (dd, J = 69.0, 8.2 Hz); ^{1}H-decoupled δ 9.38 (d, J = 69.0 Hz). HRMS (ES^+) calcd for $C_8H_{12}NO_5FP$ ($M + H^+$) m/z, 252.0432; found 252.0433. HPLC 98% purity. Anal. ($C_8H_{11}FNO_5P$): C, H, N.

[1,1-Difluoro-2-hydroxy-2-(2,2,8-trimethyl-4*H*-[1,3]dioxino[4,5-*c*]pyridin-5-yl)ethyl]phosphonic Acid Diethyl Ester (11). To a THF solution (100 mL) at −40 °C under dry nitrogen were added lithium diisopropylamide (2.0 M, 12.5 mL, 25 mmol) and *n*-BuLi (0.5 M, 1.25 mL, 2.5 mmol). Diethyl difluoromethylphosphonate (4.08 mL, 25 mmol) was then introduced to this mixture, and the reaction was stirred at this temperature for 10 min. The solution was then cooled further to −78 °C, and a solution of aldehyde **6** (5.18 g, 25 mmol) in THF (10 mL) was added dropwise to the solution containing the diethyl phosphite anion. The reaction mixture was then allowed to warm to room temperature. The solvent was evaporated, and the residue was dissolved in dichloromethane, washed with saturated aqueous $NaHCO_3$ and brine, dried (Na_2SO_4), and evaporated. Purification by column chromatography over silica gel using dichloromethane:hexane:methanol 5:5:1 gave 7.72 g (80%) of **11** as a colorless syrup. ^{1}H NMR ($CDCl_3$) δ 8.10 (s, 1H), 5.08 (ddd, J = 23.0, 4.5, 1.2 Hz, 1H), 5.03 (d, J = 16.5 Hz, 1H), 4.9 (d, J = 16.5 Hz, 1H), 4.37−4.21 (m, 4H), 2.40 (s, 3H), 1.55 (s, 3H), 1.52 (s, 3H), 1.38 (t, 3H), 1.36 (t, 3H). ^{19}F NMR ($CDCl_3$) δ −126.08 (dd, J = 304.5, 105.4 Hz), −114.59 (dd, J = 304.5, 97.2 Hz). ^{31}P NMR ($CDCl_3$, ^{1}H-decoupled) δ 7.24 (dd, J = 105.4, 96.7 Hz).

[1,1-Difluoro-2-oxo-2-(2,2,8-trimethyl-4*H*-[1,3]dioxino-[4,5-*c*]pyridin-5-yl)ethyl]phosphonic Acid Diethyl Ester (12). A mixture of alcohol **11** (3.30 g, 8.35 mmol) and MnO_2 (6.30 g, 72 mmol) in toluene (80 mL) was stirred at 60 °C overnight. After this time, the reaction mixture was filtered and the filtrate evaporated. The oily residue was purified by flash column chromatography using hexane:ethyl acetate 2:1 to 1:2 as eluent. Ketone **12**, 1.59 g (48% yield), was obtained as light yellow syrup. ^{1}H NMR ($CDCl_3$) δ 8.87 (s, 1H), 5.08 (s, 2H), 4.38−4.31 (m, 4H), 2.50 (s, 3H), 1.56 (s, 6H), 1.41 (t, J = 7.2 Hz). ^{19}F NMR ($CDCl_3$) δ −109.9 (dd, J = 94.9, 1.5 Hz). ^{31}P NMR ($CDCl_3$, ^{1}H-decoupled) δ 3.94 (t, J = 95.0 Hz). HRMS (ES^+) calcd for $C_{16}H_{23}NO_6F_2P$ ($M + H^+$) m/z, 394.1226; found 394.1226. Anal. ($C_{16}H_{22}F_2NO_6P$): C, H, N.

[1,1-Difluoro-2-(5-hydroxy-4-hydroxymethyl-6-methylpyridin-3-yl)-2-oxoethyl]phosphonic Acid Diethyl Ester (13). Compound **12** (1.59 g, 4.05 mmol) in 80% HOAc (40 mL) was stirred at 80 °C for 5 h. The solution was allowed to cool to room temperature and then evaporated to dryness. The resulting crude mixture was purified by flash column chromatography using dichloromethane:hexane:methanol 5:5:1 as eluant to give 760 mg (53%) of **13** as a white solid. ^{1}H NMR (DMSO-d_6) δ 9.73 (s, 1H), 8.03 (s, 1H), 7.81 (s, 1H), 5.07 (d, J = 14.0 Hz, 1H), 4.94 (d, J = 14.0 Hz, 1H), 4.18−4.02 (m, 4H), 3.31 (br s, 1H), 2.49 (s, 3H), 1.24 (t, J = 7.0 Hz, 3H), 1.22 (t, J = 7.0 Hz, 3H). ^{19}F NMR (DMSO-d_6) δ −118.74 (ddd, J = 303.8, 98.9, 12.7 Hz), −116.61 (ddd, J = 303.8, 98.5, 18.6 Hz). ^{31}P NMR (DMSO-d_6) δ 6.26 (td, J = 98.2, 2.7 Hz).

[(3,7-Dihydroxy-6-methyl-1,3-dihydrofuro[3,4-*c*]pyridin-3-yl)difluoromethyl]phosphonic Acid (14). Phosphonic acid diethyl ester **13** (1.05 g, 2.97 mmol) in dry acetonitrile (20 mL) was treated with excess trimethylsilyl bromide (3.13 mL, 17.8 mmol), and the reaction was stirred at rt overnight. The solvent was then evaporated, ammonium hydroxide added, and the solvent again evaporated. Chromatography over reverse phase (C-18) silica using CH_3CN:H_2O 9:1 as elution solvent gave 590 mg (60%) of **14** as a white solid. ^{1}H NMR (D_2O) δ 7.91 (s, 1H), 5.31 (d, J = 15 Hz, 1H), 5.21 (d, J = 15 Hz, 1H), 2.53 (s, 3H). ^{13}C NMR (D_2O) δ 157.19, 145.0, 143.53, 133.93, 121.8, 71.0, 14.9. ^{19}F NMR (D_2O) δ −121.80 (dd, J = 295.8, 81.2 Hz), −119.52 (dd, J = 295.8, 80.1 Hz). ^{31}P NMR (D_2O) δ 4.40 (br t, J = 80.5 Hz). HRMS (ES^+) calcd for $C_9H_{11}NO_6F_2P$ ($M + H^+$) m/z, 298.0287; found 298.0286; m/z (ES^-): 296.2 ($M-H^+$). HPLC 100% purity.

[Difluoro-(5-hydroxy-4-hydroxymethyl-6-methylpyridin-3-yl)methyl]phosphonic Acid (16). Blocked monofluorophosphonate **9** (86 mg, 0.21 mmol) was dissolved in 5 mL of THF and cooled to −78 °C under dry nitrogen. Lithium diisopropylamide (2.0 M in THF/*n*-heptane, 0.14 mL, 0.28 mmol) was then introduced, and the reaction mixture was stirred at −78 °C for 1 h. After this time, a solution of *N*-fluorobenzenesulfonimide (95 mg, 0.30 mmol) in THF (5 mL) was added, and stirring was continued at this temperature for an additional 1 h. The reaction was quenched with saturated aqueous $NaHCO_3$ and then allowed to warm to rt. The mixture was extracted with ethyl acetate, and the organic solution was dried over $MgSO_4$, filtered, and concentrated. Crude **15** obtained in this way was immediately dissolved in

HOAc:H_2O 4:1 (10 mL) and then stirred at 75 °C under nitrogen for 18 h. The volatiles were evaporated under reduced pressure, and the crude product was purified by column chromatography over silica gel using $CHCl_3$:CH_3OH:NH_4OH 65:50:25 as eluant to furnish 20 mg (35%) of **16** as a solid. ^{1}H NMR (0.1 M NaOH in D_2O) δ 7.68 (s, 1H), 4.81 (s, 2H), 2.30 (s, 3 H). ^{19}F NMR (0.1 M NaOH in D_2O) δ 100.65 (d, J = 90.1 Hz). ^{31}P NMR (0.1 M NaOH in D_2O) δ 6.00 (t, J = 90.0 Hz). HRMS (ES^+) calcd for $C_8H_{11}NO_5F_2P$ ($M + H^+$) m/z, 270.0337; found 270.034. HPLC 91% purity.

(3,7-Dihydroxy-6-methyl-1,3-dihydrofuro[3,4-*c*]pyridin-3-yl)phosphonic Acid (17). To a solution of α-hydroxyphosphonate **7** (3.5 g, 8.7 mmol) in dichloromethane (100 mL) at −8 °C under nitrogen atmosphere was added diisopropylethylamine (3.39 g, 26.2 mmol) followed by a solution of SO_3−pyridine complex (4.17 g, 26.2 mmol) in DMSO (20 mL). The yellow solution was stirred for 1.5 h at this temperature and then diluted with diethyl ether (300 mL). The organic solution was successively washed with water, saturated aqueous $NaHCO_3$, and brine and then dried ($MgSO_4$), filtered, and concentrated to give the crude blocked α-ketophosphonate as a yellow oil. This material was deprotected by stirring in HOAc:H_2O 4:1 (60 mL) at 75 °C for 18 h. After this time, the solvent was evaporated, and the resulting solid was washed with water, methanol, and finally dichloromethane to furnish 1.85 g (86%) of **17** as an off-white solid. ^{1}H NMR (0.1 M NaOH in D_2O) δ 7. 73 (s, 1H), 5.25 (dd, J = 15.0, 6.2 Hz, 1H), 5.08 (d, J = 15.0 Hz, 1H), 2.42 (s, 3H). ^{13}C NMR (0.1 M NaOH in D_2O) δ 158.1, 144.2, 143.1 (d, J = 4.0 Hz), 137.8 (d, J = 8.5 Hz), 120.5, 107.6 (d, J = 193.4 Hz), 71.4 (d, J = 5.2 Hz), 49.3, 14.9. ^{31}P NMR (0.1 M NaOH in D_2O) δ 11.1 (d, J = 6.2 Hz); ^{1}H decoupled δ 11.1 (s). HRMS (ES^+) calcd for $C_8H_{11}NO_6P$ ($M + H^+$) m/z, 248.0319; found 248.032. HPLC 99.8% purity. Anal. ($C_8H_{10}NO_6P \cdot 1.5H_2O$): C, H, N.

HPLC Assessment of Light Sensitivity. Aqueous solutions of compounds **1**, **3**, **8**, **10**, **14**, and **17** were tested for stability toward light. HPLC analysis of solutions exposed to natural light at room temperature under the conditions outlined in the general methods revealed that compounds **8**, **10**, **14**, and **17** remained unchanged after 24 h, while 42% and 70% of compounds **1** and **3**, respectively, remained after this period.

Ischemia Reperfusion Studies. This study was carried out at the William Harvey Research Ltd. (London, England) as per literature protocol.[23] Briefly, male Wistar rats (200−300 g) were anesthetized (thiopentone sodium), tracheotomized, intubated, and ventilated while maintaining the body temperature at 38 ± 1 °C. The right carotid artery was cannulated to monitor mean arterial blood pressure and heart rate. The right jugular vein was cannulated for the administration of the test compounds prior to lateral thoractomy. A snare occluder was positioned around the left anterior descending artery (LAD), and the animals were allowed to stabilize for 30 min prior to LAD occlusion. The rats were then given either saline (infusion) or test compound (bolus injection: 0.02 mmol/kg or 0.1 mmol/kg), and after 5 min, the occluder was tightened. Following 25 min of acute ischemia, the occluder was reopened to allow reperfusion for 2 h. Upon completion of the reperfusion period, the coronary artery was reoccluded, and Evans blue dye (2% w/v, 1 mL) was administered into the left ventrical (LV) via the right carotid artery cannula to identify perfused and nonperfused (area at risk of infarction, AR) regions. The Evans blue dye stains perfused myocardium while leaving the nonperfused (AR) unstained. AR was obtained as a percentage of the LV. The animals were sacrificed, and after sectioning the heart, the AR was separated form the stained nonischemic area. The AR was cut into small pieces and incubated with *p*-nitro-blue tetrazolium solution (NBT, 0.5 mg/mL) for 40 min at 37 °C. In viable myocardium, the presence of intact dehydrogenase activity leads to the formation of a dark blue formazan in the presence of NBT while necrotic regions fail to stain. The pieces were then separated based on staining and weighed to determine the infarct size (expressed as a percentage of AR).

Working Heart Perfusions. Male Sprague−Dawley rats (0.3−0.35 kg) were used in this study, and the rat hearts were cannulated and prepared for working heart perfusions as per literature protocol.[27] The working rat hearts were perfused with a modified Krebs−Henseleit solution containing calcium (2.5 mmol/L), glucose (5.5 mmol/L), palmitate (0.4 mmol/L), and 3% bovine serum albumin. Spontaneously beating hearts were employed in all perfusions and were subjected to aerobic perfusion (60 min). The test compounds were perfused at a concentration of 1 mM while the positive control DCA was perfused at a concentration of 3 mM. Fatty acid oxidation and glucose oxidation were measured simultaneously by perfusing the hearts with [^{3}H]-palmitate and [^{14}C]-glucose, respectively. The control group received no treatment. The total myocardial ^3H_2O and $^{14}CO_2$ production was measured at 10-min intervals during the aerobic period, and these data were used to calculate fatty acid and glucose oxidation, respectively.

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Supporting Information Available: HPLC purity data for compounds **8**, **10**, and **14**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Rineheart, J. F.; Greenberg, L. D. Arteriosclerotic lesions in pyridoxine-deficient monkeys. *Am. J. Pathol.* **1949**, *25*, 481−491.
(2) Wang, X.; Dakshinamurti, K.; Musat, S.; Dhalla, N. S. Pyridoxal 5-phosphate is an ATP-receptor antagonist in freshly isolated rat cardiomyocytes. *J. Mol. Cell Cardiol.* **1999**, *31*, 1063−1072.
(3) Serfontein, W. J.; Ubbink, J. B.; De Villiers, L. S.; Rapley, C. H.; Becker, P. J. Plasma pyridoxal-5-phosphate level as risk index for coronary artery disease. *Atherosclerosis* **1985**, *55*, 357−361.
(4) Ellis, J. M.; McCully, K. S. Prevention of myocardial infarction by vitamin B6. *Res. Commun. Pathol. Pharmacol.* **1995**, *89*, 208−220.
(5) Abdelmouttaleb, I.; Danchin, N.; Aimone-Gastin, I.; Namour, F.; Angioi, M.; Gelot, M.-A.; Bennani, N.; Lambert, D.; Jeandel, C.; Guéany. Homocysteine, vitamins B6, B12, folate, and risk of coronary artery disease in patients undergoing diagnostic coronary angiography. *Amino Acids* **2000**, *18*, 139−146.
(6) Robinson, K.; Mayer, E. L.; Miller, D. P.; Green, R.; van Lente, F.; Gupta, A.; Kottke-Marchant, K.; Savon, S. R.; Selub, J.; Nissen, S. E.; Kutner, M.; Topol, E. J.; Jacobsen, D. W. Hyperhomocysteinemia and low pyridoxal phosphate. *Circulation* **1995**, *92*, 2825−2830.
(7) Robinson, K.; Arheart, K.; Refsum, H.; Brattström, L.; Boers, G.; Ueland, P.; Rubba, P.; Palma-Reis, R.; Meleady, R.; Daly, L.; Witteman, J.; Graham, I. Low circulating folate and vitamin B6 concentrations risk factors for stroke, peripheral vascular disease, and coronary disease. *Circulation* **1998**, *97*, 437−443.
(8) Miner, S. E. S.; Cole, D. E. C.; Evrovski, J.; Forrest, Q.; Hutchison, S.; Holmes, K.; Ross, H. J. Pyridoxine improves endothelial function in cardiac transplant recipients. *J. Heart Lung Transplant.* **2001**, *20*, 964−969.
(9) Haque, W. Cardioprotective Phosphonates and Malonates. WO Patent 0164692.

(10) Blackburn, G. M.; Kent, D. E.; Kolkman, F. The synthesis and metal binding characteristics of novel, isopolar phosphonate analogues of nucleotides. *J. Chem. Soc., Perkin Trans. 1* **1984**, 1119−1125.
(11) Berkowitz, D. B.; Bose, M.; Pfannenstiel, T. J.; Doukov, T. α-Fluorinated phosphonates as substrate mimics for glucose 6-phosphate dehydrogenase: the CHF stereochemistry matters. *J. Org. Chem.* **2000**, *65*, 4498−4508.
(12) O'Hagen, D.; Rzepa, H. S. Some influences of fluorine in bioorganic chemistry. *J. Chem. Soc., Chem Commun.* **1997**, 645−652.
(13) Denesyuk, A. I.; Denessiouk, K. A.; Korpela, T.; Johnson, M. S. Functional Attributes of the phosphate group binding cup of pyridoxal phosphate-dependent enzymes. *J. Mol. Biol.* **2002**, *316*, 155−172.
(14) Hullar, T. L. Pyridoxal phosphate: I phosphonic acid analogues of pyridoxal phosphate. Synthesis via Wittig reactions and enzyme evaluation. *J. Med. Chem.* **1969**, *12*, 58−63.
(15) Stirtan, W. G.; Withers, S. G. Phosphonate and α-fluorophosphonate analogue probes of the ionization state of pyridoxal 5′-phosphate (PLP) in glycogen. *Biochemistry* **1996**, *35*, 15057−15064.
(16) Kim, Y.-C.; Camaioni, E.; Ziganshin, A. U.; Ji, X.-D.; King, B. F.; Wildman, S. S.; Rychkov, A.; Yoburn, J.; Kim, H.; Mohanram, A.; Harden, T. K.; Boyer, J. L.; Burnstock, G.; Jacobson, K. A. Synthesis and structure−activity relationships of pyridoxal-6-arylazo-5′-phosphate and phosphonate derivatives as P2 receptor antagonists. *Drug Dev. Res.* **1998**, *45*, 52−66.
(17) Dhalla, N. S.; Sethi, R.; Dakshinamurti, K. Treatment of Cardiovascular and Related Pathologies. US Patent 6,043,259.
(18) Korytnyk, W.; Ikawa, M. Synthesis of vitamin B_6 analogues. *Methods Enzymol.* **1970**, *18A*, 524−566.
(19) Brooks, H. G., Jr.; Laasko, J. W.; Metzler, D. E. Synthesis of vitamin B_6 derivatives 1. Preparation of some isopyridoxamine derivatives from isopyridoxal. *J. Heterocycl. Chem.* **1966**, 126−128.
(20) Pudovik, A. N.; Konovalova, I. V. Addition reactions of esters of phosphorous III acids with unsaturated systems. *Synthesis* **1979**, 81−96.
(21) Ferrari, R.; Pepi, P.; Ferrari, F.; Nesta, F.; Benigno, M.; Visioli, O. Metabolic Derangement in Ischemic Heart Disease and its Therapeutic Control. *Am. J. Cardiol.* **1998**, *82* (5A), 2K−13K.
(22) Dellborg, M.; Herlitz, J.; Risenfors, M.; Swedberg, K. Electrocardiographic assessment of infarct size: comparison between QRS scoring of 12-lead electrocardiography and dynamic vector cardiography. *Int. J. Cardiol.* **1993**, *40*, 167−172.
(23) Zacharowski, K.; Blackburn, B.; Thiemermann, C. Ranolazine, a partial fatty acid oxidation inhibitor, reduces myocardial infarct size and cardiac troponin C release in the rat. *Eur. J. Pharmacol.* **2001**, *418*, 105−110.
(24) Kantor, P. F.; Dyck, J. R. B.; Lopaschuk, G. D. Fatty acid oxidation in the reperfused ischemic heart. *Am. J. Med. Sci.* **1999**, *318*, 3−14.
(25) Lopaschuk, G. D. Optimizing cardiac energy metabolism: a new approach to treating ischemic heart disease. *Eur. Heart J. Suppl.* **1999**, *1 (Suppl. O)*, O32−O39.
(26) Bersin, R. M.; Stacpoole, P. W. Dichloroacetate as metabolic therapy for myocardial ischemia and failure. *Am. Heart J.* **1997**, *134*, 841−855.
(27) Lopaschuk, G. D.; Barr, R. L. Measurement of fatty acid and carbohydrate metabolism in isolated working rat hearts. *Mol. Cell Biochem.* **1997**, *172*, 137−147.
(28) Lopaschuk, G. D.; Wambolt, R. B.; Barr, R. L. An imbalance between glycolysis and glucose oxidation is a possible explanation for the detrimental effects of high levels of fatty acid during aerobic reperfusion of ischemic hearts. *J. Pharmacol. Exp. Ther.* **1993**, *264*, 135−144.

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