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Prodrugs of Pioglitazone for Extended-Release (XR) Injectable Formulations

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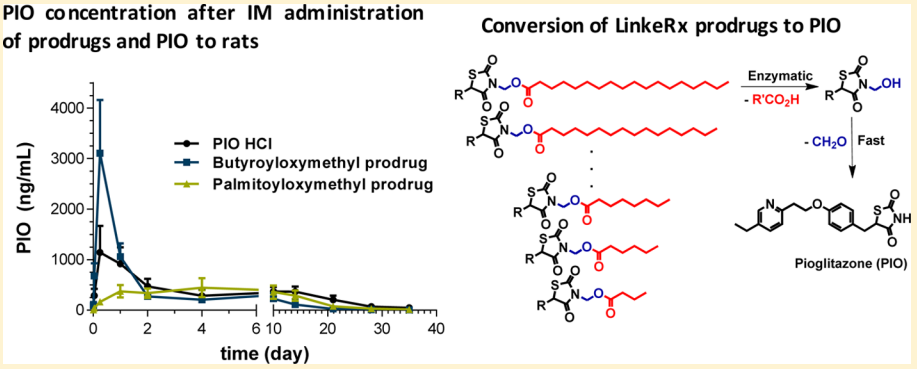
Supporting Information



ABSTRACT: *N*-Acyloxymethyl derivatives of pioglitazone (PIO) have been prepared and characterized as model candidates for extended-release injectable formulations. All PIO derivatives prepared are crystalline solids as determined by powder X-ray diffraction, and the solubility in aqueous media is below 1 μM at 37 °C. The melting points steadily increase from 55 °C, for the hexanoyloxymethyl derivative, to 85 °C, for the palmitoyloxymethyl derivative; inversely, the solubilities in ethyl oleate decrease as a function of increasing acyl chain length. The butyroyloxymethyl ester has a higher melting point and a lower solubility in ethyl oleate than expected from the trend. The ^{13}C solid-state NMR spectra of the PIO homologues between the hexanoyloxymethyl derivative and stearoyloxymethyl derivative suggest a common structural motif with the acyl chains exchanging between two distinct conformations, and the rate of exchange is slower for longer chain derivatives. The butyroyloxymethyl derivative is efficiently converted to PIO in *in vitro* rat plasma with a half-life of <2 min at 37 ° C, while the rate of enzymatic cleavage in rat plasma decreases as the ester chain length increases for the longer acyloxymethyl derivatives. The concentration of PIO in plasma increases rapidly, or "spikes," in the hours following intramuscular (IM) injection of either the HCl salt or the butyroyloxymethyl derivative. In contrast, the more lipophilic palmitoyloxymethyl derivative provides slow growth in the PIO concentration over the first day to reach levels that remain steady for 2 weeks. On the basis of its *in vivo* pharmacokinetic profile, as well as material and solubility properties, the PIO palmitoyloxymethyl derivative has potential as a once-monthly injectable medication to treat diabetes.

KEYWORDS: *pioglitazone prodrugs, extended-release, prodrugs, N-acyloxy derivatives, solid-state NMR, long acting injectable (LAI), diabetes*

INTRODUCTION

Pioglitazone (PIO) is the leading PPARγ agonist used to treat Type II Diabetes Mellitus with superior efficacy and tolerability over other thiazolidinediones;[1,2] however, its use is limited by safety concerns.[3,4] An extended-release injectable formulation of PIO could provide a reduced peak-to-trough ratio in drug plasma concentrations, which may provide improved safety and tolerability[5] and improved bioavailability by mitigating first-pass metabolism of the oral therapy.[6] Extended-release formulations of PIO utilizing polymer microspheres to control the rate of release of the active drug have been reported.[7] Since the daily dose of PIO is considerable (up to 45 mg), the total mass of injection for a once-monthly injectable formulation is a concern. As a result of the limited drug-loading achievable in microspheres, alternative extended-release formulations of PIO are desired. We report herein the synthesis and characterization of *N*-acyloxymethyl prodrug derivatives of PIO that have potential to provide extended release of PIO from an aqueous suspension of crystalline material.

There are a number of prodrugs utilized in marketed extended-release injectable formulations of small-molecule drugs, formulated as oil solutions (haloperidol decanoate, fluphenazine decanoate, and fluphenazine enanthate) or as a

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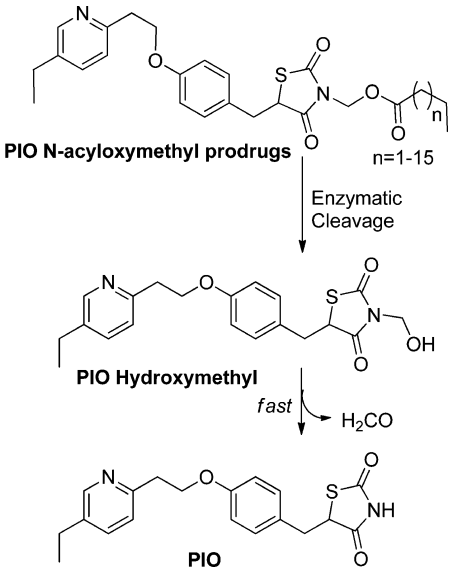
nanocrystal aqueous suspension[8] (Invega Sustenna (paliperidone palmitate)).[9] In these examples, a hydroxyl functional group in the drug molecule has been esterified with a lipophilic fatty acid to produce a prodrug derivative with desired solubility properties.

Recently, LinkeRx technology has been developed to provide crystalline low-solubility prodrugs from drugs, such as PIO, that lack hydroxyl functional groups.[10,11] These prodrug modifications allow for engineering the material and physical chemical properties that are required for extended-release injectable formulations.[12] The desired formulation is a simple aqueous suspension of crystalline prodrugs. The ideal mechanism of release is rate-limiting dissolution of the prodrug from the depot followed by rapid absorption of the prodrug into systemic circulation and efficient enzymatic cleavage, to provide sustained, efficacious concentrations of the active drug and relatively low circulating concentrations of the prodrug derivative (Scheme 1). This has been accomplished with the current phase 3 clinical development candidate, aripiprazole lauroxil, a prodrug of the partial D2 agonist, aripiprazole.[10,13]

Scheme 1. Conversion of the LinkeRx Derivatives to PIO



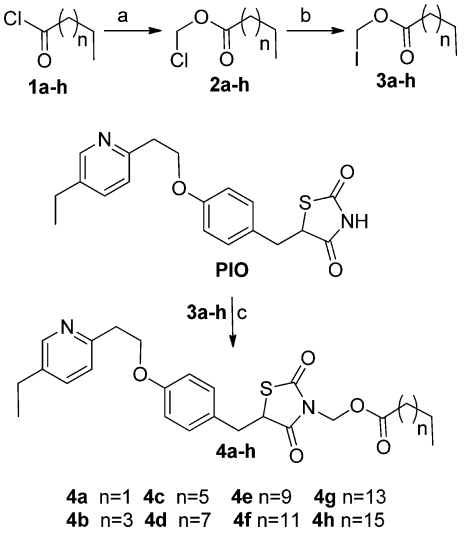
The thiazolidinedione ring of PIO has been identified as a potential site for prodrug modification; this ring system is considered an imide-type NH acid as defined by Stella.[14] Bundgaard and Stella demonstrated the utility of *N*-acyloxyalkyl or phosphoryloxyalkyl modifications of weak NH acids to provide bioreversible derivatives with modified physical chemical properties.[15] One example of a marketed drug that utilizes this prodrug approach is fosphenytoin, the soluble phosphoryloxymethyl derivative of phenytoin, a therapy for epilepsy.[16,17] *N*-Acyloxylmethyl derivatives of imide-containing drugs are enzymatically cleaved to release *N*-hydroxymethyl intermediates that release formaldehyde and the parent drug.[18,19] The rate of dehydroxymethylation of the intermediate is dependent on the pK_a of the parent drug as well as pH; for example, the pK_a of phenytoin is 8.3 and the measured half-life of phenytoin hydroxymethyl derivative at pH 7.4 and 37 °C is one second.[20,21] On the basis of the reported pK_a of PIO (5.95),[22] the predicted half-life of the hydroxymethyl−PIO derivative at pH 7.4 and 37 °C is less than one second.

A series of acyloxymethyl derivatives of PIO with varying chain lengths of the acyl groups have been synthesized and evaluated for their thermodynamic and material properties as well as their extended-release profiles from IM injection in rats.

EXPERIMENTAL SECTION

Synthesis of the Prodrugs. The *N*-acyloxymethyl derivatives of PIO were synthesized in a three-step process and were isolated as free bases (Scheme 2).

Scheme 2. Synthesis of the PIO *N*-Acyloxymethyl Derivatives[a]



[a]Reagents and conditions: (a) $ZnCl_2$, paraformaldehyde, acetonitrile, 0 °C → ambient temperature, 1 h, 90 °C for 15 h, 70% yield; (b) NaI, acetonitrile, dark, ambient temperature, 15 h, 55% yield; (c) K_2CO_3, dimethylformamide, ambient temperature 15−48 h, 21−78% yield.

General Procedure for the Synthesis of Chloromethyl Esters (Step 1).[23] Acid chloride (one equiv) was added dropwise to an acetonitrile solution of paraformaldehyde (one equiv) and anhydrous zinc chloride (0.5 equiv) at 0 °C under argon. After the addition was complete, the reaction mixture was warmed to 25 °C and stirred for 1 h, then heated to 90 °C for 15 h. The solid was filtered off and washed with dichloromethane; the filtrate was concentrated under vacuum at 37 °C to provide chloromethyl ester, which was used directly (without purification) in the next step.

General Procedure for the Synthesis of Iodomethyl Ester (Step 2).[24] A solution of chloromethyl ester (one equiv) in acetonitrile (86 mL) was treated with sodium iodide (three equiv). The flask was covered in aluminum foil and stirred at 25 °C for at least 15 h. The reaction mixture was partitioned between dichloromethane and water, and the aqueous layer was extracted with dichloromethane. The combined organics were washed with saturated aqueous $NaHCO_3$, 10% aqueous sodium sulfite solution, and brine, then dried with sodium sulfate and concentrated to give the iodomethyl ester as a yellow oil, which was used in the alkylation step without further purification.

General Procedure for the Synthesis of PIO Acylalkoxyl Derivatives (Step 3). A solution of PIO (one equiv) in dimethylformamide was treated with dry K_2CO_3 (three equiv) at 25 °C. After 40 min, a solution of iodomethyl ester (two equiv) was added dropwise. The reaction mixture was stirred for at least 15 h until alkylation was complete, then dumped

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into water and extracted with ethyl acetate. The combined organic layers were dried with sodium sulfate and concentrated under vacuum. The product was purified by flash chromatography on silica gel.

Recrystallization. Samples of **4b**−**h** were recrystallized from isobutyl acetate/heptane or isobutyl acetate/pentane and dried under vacuum or air-dried. The growth of single crystals was attempted by slow evaporation from several solvents. Unfortunately, all attempts at crystallization produced only very small, fine needles that were unsuitable for single crystal X-ray diffraction.

Microscopy. An Olympus BX51 Reflected Polarized Light Microscope was used to determine crystal morphology and characterize the particle size of the suspensions.

Powder X-ray Diffraction (PXRD). PXRD was performed on a Rigaku Miniflex 2 diffractometer (Rigaku/MSC, Woodlands, TX) with a Si zero-background holder with ϕ rotation and Cu Kα radiation operating at 30 kV/15 mA. Data were processed using Eva 2 (Bruker).

Solid-State NMR. ^{13}C cross polarized-magic angle spinning NMR (^{13}C CP/MAS NMR) spectra were obtained on a Varian NMR operating at 399.746 MHz for ^{1}H and 100.527 MHz for ^{13}C. Spectra were collected using a 4 mm T3-HFX probe tuned to ^{13}C and ^{1}H. All samples were spun at 10 kHz with temperature set at 10 °C unless otherwise stated. ^{13}C CP/MAS spectra were collected using the tangentially ramped cross-polarization pulse sequence (tancpx) included as part of SolidsPack library of pulse sequences for use with the Vnmrj 3.1a spectrometer operating software by Agilent Technologies. The power levels were adjusted to provide $\pi/2$ = 2.5 μs for both channels. Cross-polarization and decoupling parameters were optimized using glycine, and the spectra were referenced to the carbonyl peak of glycine at 176.5 ppm. The delay time between transients was set for at least 1.2 times the value of T1 for ^{13}C CP/MAS spectra, and for >5 times T1 during measurement of ^{1}H or ^{13}C T1 values. Spectral overlays were produced using IGOR Pro 6.05 by Wavemetrics, Inc.

Thermal Analysis. Differential scanning calorimetry (DSC) curves were acquired using a TA Instruments Q1000. Typically, 1−2 mg of sample were weighed into an aluminum pan (Tzero), sealed with a pinhole lid and heated at 10 °C/min from 25 to 200 °C. Melting points are reported as the peak temperature of the melting endotherm. Thermal gravimetric analysis (TGA) was performed on a TA Instruments TGA Q500. Samples of 5−10 mg were heated at 10 °C/min from 25 to 250 °C. The data were processed using Universal Analysis 2000 Version 4.3A.

General Procedure for Solubility Measurements in Aqueous Media. Suspensions of the prodrugs (5 mg/mL) in PBS were stirred at 37 °C for 3−4 days. Daily, 1 mL aliquots were filtered; the filtrate diluted with MeCN or MeOH (×2) and analyzed by HPLC to determine the amount of prodrug dissolved. After 3−4 days, the remaining suspension was filtered and the solid analyzed by PXRD to confirm preservation of the crystal; and after dissolution with MeCN or MeCN/THF, it was analyzed by HPLC to confirm purity after the experiment.

General Procedure for Solubility Measurements in Ethyl Oleate. Suspensions of the prodrugs in ethyl oleate were stirred at room temperature for 2 days. The samples were filtered and the filtrate diluted with MeCN/THF (×10 or ×100) and analyzed by HPLC to determine the amount of prodrug dissolved. The remaining solids were analyzed by PXRD to confirm preservation of the crystalline form during the experiment.

ClogP. The partition coefficients (logP) were calculated using ChemBioDraw Ultra version 12.0 from CambridgeSoft Specific algorithms for calculating logP from fragment-based methods developed by the Medicinal Chemistry Project and BioByte.

General Procedure for Aqueous Hydrolysis Measurements. The aqueous hydrolysis of the prodrugs was performed in the presence of a cosolvent (20% MeCN or DMSO) because of the very low solubility of the prodrugs in neat aqueous media. Then, 0.05−0.1 mM solutions of the prodrugs **4a**, **4e**, **4g**, and PIO were prepared in PBS/MeCN 8:2 and/or PBS/DMSO 8:2 and incubated at 37 °C. Typically, aliquots (0.010 mL) were sampled at different time points and immediately injected in the HPLC with UV detection and, for some experiments, with MS detection. Substantial precipitation was observed for **4e**, **4g**, and PIO even in the presence of 20% cosolvent. Therefore, the half-life could only be calculated for **4a**.

General Procedure for Rat Plasma Incubation. Stock solutions of the prodrugs were prepared in acetonitrile or acetonitrile/THF. A 0.010 mL aliquot of the stock was spiked into 1 mL of rat plasma and incubated at 37 °C. The concentration of the prodrugs in rat plasma was 0.250 mM, when HPLC−UV was used for sample analysis or 0.003 mM when HPLC−MS/MS was used. Typically, aliquots (0.050 mL) were sampled at different time points and immediately dispensed into Eppendorf tubes containing 0.200 mL of acetonitrile to precipitate the protein. The tubes were centrifuged, and the supernatant was isolated and analyzed by HPLC−UV or HPLC−MS/MS. The peak areas corresponding to the prodrugs were plotted against time, and the data were fitted to a first-order monoexponential decay where the rate constant and the half-life were determined from the slope.

Rat IM PK Dosing. The studies were completed under protocols approved by the Alkermes Institutional Animal Care and Use Committee and were conducted in accordance with the Institute of Laboratory Animal Resources.[25] Each compound was suspended using aseptic techniques at a concentration of 66.7 mg/mL (PIO equivalents) in a vehicle containing 2% sodium carboxymethyl cellulose (CMC), 0.2% polysorbate 20, and PBS buffer, 302 mOsm/kg pH 6.7. The vehicle was autoclaved before use at 121 °C for 20 min. There were six rats in each group. A 0.3 mL suspension dose (20 mg PIO equivalents) was injected immediately into the hind limb muscles of rats after anesthesia with isoflurane using a single smooth push with a 1 mL syringe with a 23-gauge, one-inch needle. Time points: 0.25, 1, and 6 hours and 2, 4, 7, 10, 14, 21, and 35 days after administration. Approximately 0.250 mL of whole blood was collected at each sampling time through day 14 and 0.450 mL of whole blood for each time point after day 14. All blood samples were collected via a lateral tail vein after brief anesthesia with Isoflurane. A 27G half-inch needle and 1 mL syringe without an anticoagulant was used for blood collection. Once collected, whole blood was transferred to tubes containing K2-EDTA, inverted 10−15 times, and placed on ice. The tubes were then centrifuged for 2 min at >14,000g (11,500 rpm using an Eppendorf centrifuge) at 4−8 °C to separate plasma. The plasma samples were transferred to chilled (4−8 °C) plain tubes (96-well plate) and stored frozen at −70 °C.

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PIO concentrations in plasma samples were analyzed by liquid chromatography−mass spectroscopy (HPLC−MS/MS) using appropriate parameters for each compound. Half-life, maximal concentration, and AUC were calculated by non-compartmental analysis using WinNonlin version 5.2 software (Pharsight, St. Louis, MO) based on measured PIO concentrations through day 35.

RESULTS AND DISCUSSION

Structural Characterization of the Prodrugs by PXRD and Solid-State NMR. All of the PIO derivatives prepared were crystalline solids as determined by PXRD (Figure 1). The

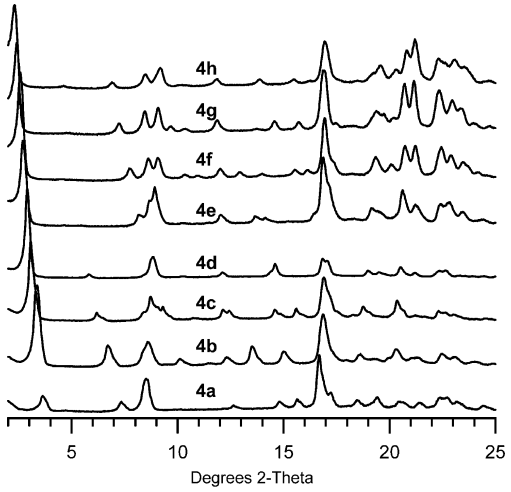


Figure 1. PXRD pattern at room temperature for **4a−h**.

structures were characterized with PXRD and ^{13}C CP/MAS NMR studies that suggest compounds **4a−h** pack with similar structural motifs where the lattice expands for longer chain lengths. The lowest angle diffraction peak decreased from 3.4 to 2.9° 2θ with increasing chain length, indicating an expansion of the longest axis of the unit cell. However, many of the peaks, or clusters, change very little with extending chain length, suggesting that the overall arrangement of atoms in the lattice is similar. ^{13}C CP/MAS NMR spectroscopy was used as another probe, as chemical shifts are sensitive to the local environment of atoms within the crystal lattice.

The chemical shift values between 50 and 165 ppm in the ^{13}C CP/MAS NMR spectra of the PIO derivatives (Figure 2)[26−28] correspond to the linker and the majority of the core PIO structure. These peaks remain identical throughout the series with no changes in the number of carbon peaks, suggesting that crystal packing motif is similar for all of the derivatives between **4b** and **4h**. However, there are some changes in the peaks and chemical shifts that correspond to the acyl chain and carbonyls, which are suggestive of differences in the local environment of these groups within the crystal lattices of the different derivatives.

The changes in the peaks corresponding to the acyl chain, 10−40 ppm, have been explored as a function of temperature, and they appear to be changes in the acyl chain mobility within the crystal structure. Figure 3 shows temperature-dependent changes in the alkyl region (10−40 ppm) of **4e** (lauroyloxymethyl) and **4g** (palmitoyloxymethyl), as representatives of derivatives containing longer acyl chains (**4e**, **4f**, **4g**, and **4h**). The spectra at 0 °C show two pairs of peaks between 14 and 17

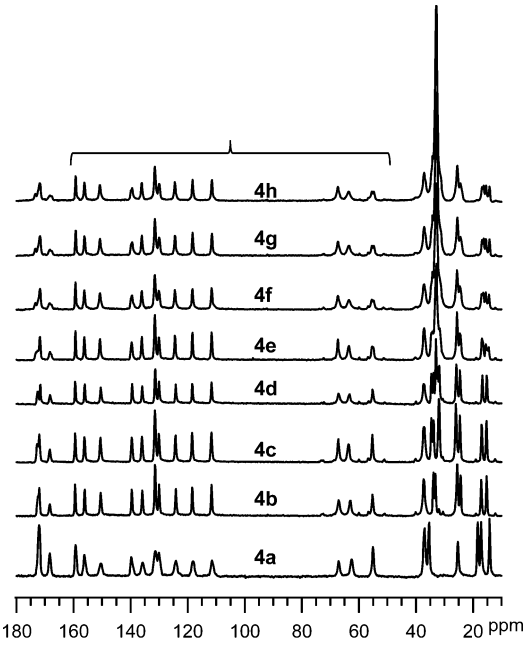


Figure 2. ^{13}C CP/MAS NMR spectroscopy on a Varian NMR 400 MHz using a 4 mm probe for **4a−h** at 10 °C.

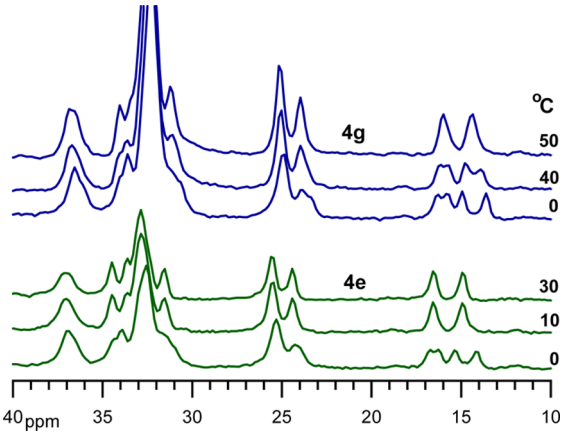


Figure 3. Temperature dependence in the alkyl region of the ^{13}C CP/MAS NMR spectra for **4e** and **4g**.

ppm, with each pair representing a methyl group. Each of these pairs coalesces, sharpening to a single peak at higher temperatures.

The coalescence temperature occurs between 0 and 10 °C for **4e** (lauroyloxymethyl), but in the higher range of 30−50 °C for **4g** (palmitoyloxymethyl). There are no detectable thermal transitions in the DSC curves at these temperatures, which is consistent with an increasing rate of motion with temperature rather than a sudden change from static to free rotation (or a very low heat capacity transition). Overall, the solid-state data for **4b−h** analogues suggest a common structural motif with the alkyl chains exchanging between two distinct conformations and the rate of exchange being slower for longer chains.

Solubility and Melting Behavior of the Prodrugs. The solubility of all of the derivatives in aqueous media is very low, below 1 μm, but a significant solubility difference is observed in ethyl oleate (EO), a model solvent for assessing solubility in lipid milieu. The overlay of the melting point and EO solubility curves as a function of acyl chain length shows an inverse

correlation (Figure 4). High oil solubility corresponds to low melting solids and vice versa. This inverse correlation between lipid solubility and melting points has been reported previously.[16,29]

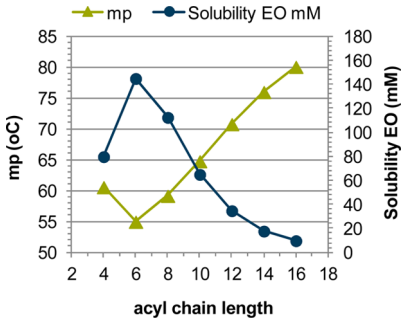


Figure 4. Solubility of **4a**–**4g** in ethyl oleate at room temperature and melting point vs acyl chain length.

The curves follow a trend of increasing melting point and decreasing solubility with increasing chain length beginning with the hexanoyloxymethyl ester. The butyroyloxymethyl ester, **4a**, breaks the trend, melting at higher temperature than expected and having a lower solubility in ethyl oleate (EO). The heat of fusion increases linearly with the acyl chain length from 165 to 200 mJ/mol for **4a**–**e**, and from 195 to 205 mJ/mol for **4f**–**h**. The palmitoyloxymethyl ester, **4g**, one of the most lipophilic derivatives, has the highest melting point and lowest solubility in the series.

Prodrug Stability in Rat Plasma. The rate of *in vitro* conversion of the PIO derivative to PIO was determined in a biological matrix known to express carboxylesterase enzymes.[30−32] The rate of prodrug conversion in rat plasma at 37 °C is represented as the half-life derived from monitoring the disappearance of prodrugs over time. Data at 37 °C were fitted to a first-order kinetic equation for prodrug loss and appearance of PIO. The rate of enzymatic cleavage decreases as the ester chain increases, with fatty acid chains shorter than six carbons showing rapid conversion and half-lives less than 2 min (Table 1).

Table 1. Half-Life of Prodrug Hydrolysis in Rat Plasma at 37 °C and Calculated logP

	half-life (minutes)	ClogP
4a (butyroyloxymethyl)	<2[a,b]	3.78
4b (hexanoyloxymethyl)	<2[a]	4.84
4e (lauroyloxymethyl)	20[a], 8[b,c]	8.01
4f (myristoyloxymethyl)	~60[a]	9.07
4g (palmitoyloxymethyl)	690[b,d]	10.13

[a]HPLC–UV. [b]HPLC–MS/MS. [c]Deviation from linearity at high conversion (>50%). [d]Extrapolated, calculated from slope.

The half-life for the cleavage of lauroyloxymethyl derivative, **4e**, is 20 min. The PIO derivatives with acyl chains longer than 14 carbons (myristoyloxymethyl) appear to be resistant to the esterase enzymes as the half-lives exceed 400 min for palmitoyloxymethyl. This dependence of the plasma rate of hydrolysis on the length of the acyl chain has been previously observed for lipophilic prodrugs.[33]

The absence of measurable levels of the intermediate *N*-hydroxymethyl–PIO derivative was confirmed by HPLC–MS/MS, which is consistent with the expected high instability of this intermediate. On the basis of the reported pK_a of PIO (5.95)[22] and hydroxide ion activity (aOH$^-$) at pH 7.4 and 37 °C, which is 6.02 × 10^{-7} M, the half-life of the hydroxymethyl–PIO derivative can be predicted to be 0.01 s (= 0.693/k_{obs}), where k_{obs} = 3883 min^{-1} (= k_1 × aOH$^-$), and the apparent hydroxide ion catalytic rate constant is k_1 = 6.45 × 10^9 min^{-1} M^{-1} (= antilog(14.4−0.77 × 5.95)).[20,21]

Prodrug Stability in Aqueous Media. The low aqueous solubility of the long-chain prodrugs (hexanoyloxymethyl derivative and longer) precludes stability measurements in aqueous buffer. As a result, the butyroyloxymethyl **4a** derivative is the only derivative for which the stability was determined in phosphate-buffered saline pH 7.5 (PBS)/DMSO 8:2 at 37 °C. In Figure 5, the disappearance of **4a** and the formation of its degradants and PIO are plotted against time.

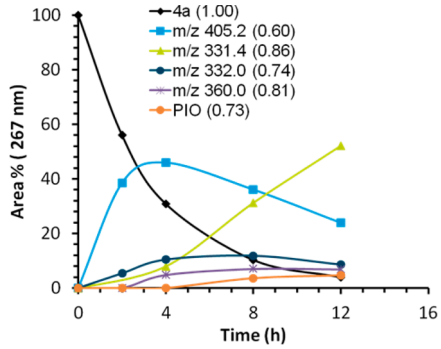
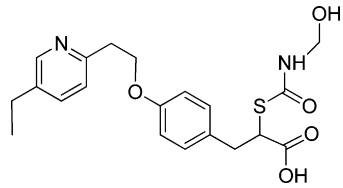


Figure 5. Area % vs time for **4a** and its degradants in PBS/DMSO 8:2 at 37 °C. The mass/charge ratio (m/z) and relative retention times (RRT) for major peaks in the HPLC–UV–MS chromatograms are indicated in the legend.

The butyroyloxymethyl prodrug **4a** does not convert to PIO (MW 356.4) in this medium but mainly to a single product with a molecular weight of 404, as measured by HPLC–MS. The proposed structure for the breakdown product is shown in Scheme 3 and corresponds to the hydrolysis of both the ester

Scheme 3. Proposed Structure for the Main Breakdown Product of 4a in PBS/DMSO 8:2 at 37 °C



group and of the thiazolidine-2,4-dione ring. Further decomposition of this product to lower molecular weight byproducts is observed throughout the experiment. PIO has low solubility in the reaction media, so to confirm that PIO was not formed in the hydrolysis of **4a**, MeOH was added after 18 h to redissolve any PIO precipitate. Analysis of resulting methanol solution confirmed the absence of a significant amount of PIO. A similar result was obtained after incubating at 37 °C **4a** in PBS/MeCN 8:2. The peak areas corresponding to **4a** were plotted against time, and the data were fitted to a first-order monoexponential decay where the rate constant and the half-life, 3.7 h, were calculated from the slope. The half-life for the **4a** degradation in buffer is significantly slower than the

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conversion to PIO in rat plasma (<2 min) at 37 °C; therefore, it is unlikely that this nonproductive degradation occurs *in vivo* to a significant extent.

Pharmacokinetics of the Prodrugs in Rats after IM Injection. After establishing that the PIO derivatives have the material and solubility properties to allow for formulation as aqueous suspensions, along with enzymatic activity to act as productive prodrugs of PIO, we evaluated the prodrugs *in vivo* in rat pharmacokinetic studies. One short-chain derivative (**4a**) and one long-chain derivative (**4g**) were dosed as IM injections of aqueous suspensions of crystalline material. The resulting plasma concentrations of PIO were measured and compared to the PIO plasma concentrations achieved when a similar formulation of PIO HCl is dosed intramuscularly. As shown in Figure 6, PIO dosed as the hydrochloride salt and the

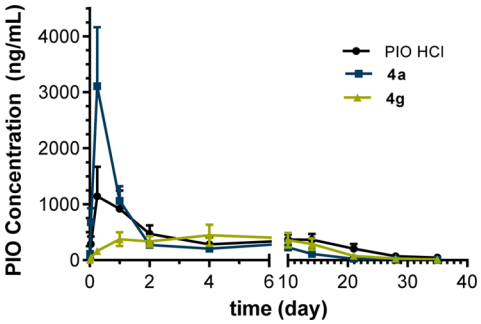

Figure 6. Mean PIO concentration (linear scale) measured following IM administration of PIO acyloxymethyl prodrugs **4a** (butyroyloxymethyl) and **4g** (palmitoyloxymethyl) and PIO HCl suspensions to rats (n = 6). Dose: 20 mg of PIO equivalents (0.3 mL of 66.7 mg/mL (PIO equivalents) in 2% CMC, 0.2% Tween 20, and PBS buffer, 302 mOsm/kg pH 6.7).

butyroyloxymethyl derivative (**4a**) show rapid absorption on day 1, followed by distribution and elimination. The highly lipophilic palmitoyloxymethyl derivative, **4g**, exhibited the slowest release of PIO with a significantly delayed T_{max}.

The comparison of the AUC_{0-t} achieved with equivalent doses of **4a** and **4g** with PIO HCl demonstrated that both derivatives efficiently converted to PIO *in vivo* (Table 2). The

Table 2. Calculated Pharmacokinetic Parameters for PIO HCl, 4a and 4g

	PIO AUC $_{0-t}$ (ng·day/mL)	t_{max} (d)
PIO HCl	9270 (1910)	0.3
4a (butyroyloxymethyl)	5970 (888)	0.3
4g (palmitoyloxymethyl)	6760 (1110)	4.0

absorption profile for PIO after PIO HCl dosing is likely influenced by the conversion of the PIO HCl salt to the lower solubility free base form at neutral pH in the muscle. The short-chain butyloxymethyl modification appears to accelerate absorption, with **4a** providing a higher early C_{max} relative to both PIO and the palmityloxymethyl derivative. Particle size does not appear to be a key factor in defining the rate of absorption since microscopy studies show that the particle size of **4a**, which provides a high and early C_{max}, is significantly larger than the particle size of **4g**. While the aqueous solubility of **4a** and **4g** is lower than PIO free base (3.5 μM in PBS), the solubility in ethyl oleate, as a model lipid, is much higher for the prodrugs, especially for the butyroyloxymethyl **4a** (80 mM vs <30 μM for PIO freebase). The low heat of fusion coupled with high-lipid solubility of **4a** are contributing to its rapid absorption in rat. The longer-chain **4g** derivative shows slower release of PIO with a delayed t_{max} of 4 days.

■ CONCLUSIONS

Use of the methyleneoxy linker group installed on a weakly acidic thiazolidine-2,4-dione ring creates the opportunity to make reversible fatty acid ester prodrugs of PIO. Such prodrugs are crystalline, have low aqueous solubility, and provide sustained release as aqueous suspensions following intramuscular injections. The *N*-acyloxymethyl prodrugs of PIO appear to pack in a common motif in the crystal lattice regardless of acyl chain length, where the long axis of the lattice expands with increasing the chain length. Longer acyl chains become simultaneously more resistant to melting and less soluble in oily solvents, consistent with a stronger packing efficiency. Likewise, the molecular motions in the lattice slow with increasing acyl chain length as shown by ^{13}C CP/MAS NMR. The rate of cleavage in rat plasma at 37 °C is rapid for derivatives with acyl chain lengths up to lauroyloxymethyl but steeply decreases for longer-chain derivatives. Rat plasma concentrations of PIO following an IM injection of the palmitoyloxymethyl ester of PIO show extended release with no spike in plasma PIO concentration; while an IM injection of the shorter-chain butyroyloxymethyl derivative releases PIO faster than PIO HCl. This study provides a proof-of-concept that LinkeRx technology can be utilized to tune key physicochemical properties of a drug that contribute to rate of absorption from a depot in tissue. The palmitoyloxymethyl prodrug has the potential to be developed as a once-monthly injectable formulation based on its reduced aqueous solubility and absorption rate. On the basis of its IM pharmacokinetic profile in rats, this novel PIO prodrug has the potential to provide a very low peak/trough ratio in humans at steady state, which is a hallmark of well-designed extended-release injectables.

■ ASSOCIATED CONTENT

Supporting Information

^{1}H and ^{13}C NMR spectra, HPLC−MS data, and DSC curves for all compounds are included, as well as the chromatograms corresponding to the stability in aqueous media of **4a**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS

PIO, pioglitazone; PBS, phosphate buffer saline pH 7.5; PXRD, power X-ray diffraction; HPLC-MS, high-performance liquid chromatography−mass spectrometry; HPLC−UV, high-performance liquid chromatography/ultraviolet spectrophotometry; DSC, differential scanning calorimetry; LC−MS/MS, liquid chromatography-tandem mass spectrometry; ^{13}C CP/MAS NMR, ^{13}C cross polarized−magic angle spinning nuclear magnetic resonance

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