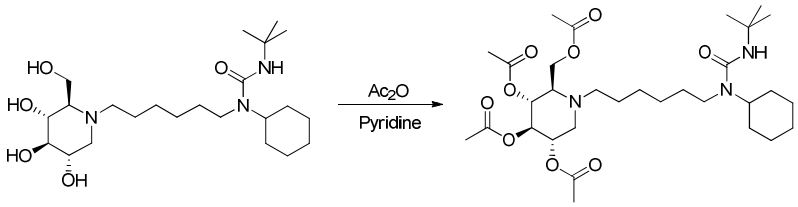
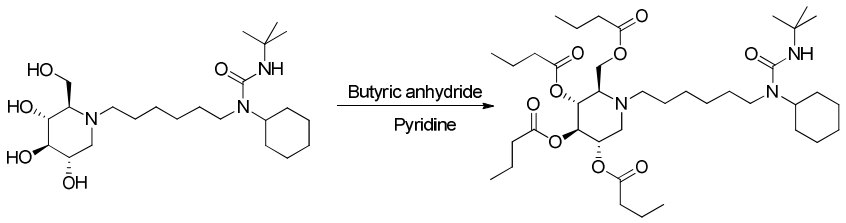
**Experimental section**

$^1$H NMR spectra were recorded on a 300 MHz INOVA VARIAN spectrometer. Chemical shifts values are given in ppm and referred as the internal standard to TMS (tetramethylsilane). The peak patterns are indicated as follows: s, singlet; d, doublet; t, triplet; q, quadruplet; m, multiplet and dd, doublet of doublets. The coupling constants (*J*) are reported in Hertz (Hz). Purity was determined on the Agilent 1200 Series/1260 Infinity/6120 Quadrupole LC/MS instrument, equipped with Agilent Eclipse XDB-C18, 5 mm/4.6 x 150 mm and UV detection at 254 nm. Eluent system was: A (Water, 0.1% Formic acid) and B (Methanol, 0.1% Formic acid); flow rate = 1 mL/min; Method: 20%B/3 min, 20-95%B/3 min, 95%B/6 min, 95-20%B/1 min Silica gel column chromatography was performed over silica gel 100-200 mesh, and the eluent was a mixture of ethyl acetate and hexanes, or mixture of methanol and ethyl acetate. All the tested compounds possess a purity of at least 95%. Pharmacokinetic Study was performed by SRI Biosciences. The *in vivo* PK study was reviewed and approved by the Institutional Animal Care and Use Committee of SRI, and carried out in accordance with the recommendations and guidelines of the National Research Council (NRC) Guide for the Care and Use of Laboratory Animals, 8th edition (2011) and the Animal Welfare Standards incorporated in 9 CFR Part 3, 1991. Caco-2 and stability tests were conducted in Pharmaron. HRMS data were measured in the Mass Spectrometry & Proteomics Facility of University of Notre Dame.

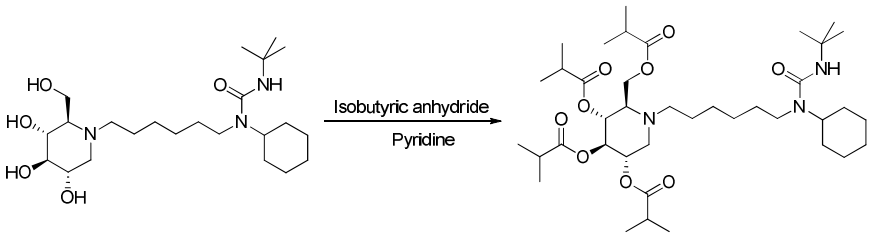



**(2R,3R,4R,5S)-2-(acetoxymethyl)-1-(6-(N-cyclohexylpivalamido) hexyl)piperidine-3,4,5-triyl triacetate 7**. 3-(tert-butyl)-1-cyclohexyl-1-(6-((2R,3R,4R,5S)-3,4,5-trihydroxy-2-(hydroxymethyl)piperidin-1-yl)hexyl)urea **6** (100 mg, 0.22 mmol) was dissolved in pyridine (1.0 mL) and treated with acetic anhydride (1.0 mL). The mixture was stirred at room temperature for overnight. Toluene was added to dilute the mixture and concentrated. The residue was dissolved in ethyl acetate and washed with saturated potassium hydrogen sulfate, saturated sodium bicarbonate, brine, and concentrated. Purification on silica gel with a gradient of ethyl acetate : hexanes from 0 : 1 to 4 : 6 gave a sticky oil (115.2 mg, 84%). $^1$H NMR (300 MHz, $CDCl_3$): δ 5.12-4.91 (m, 3H), 4.21-4.10 (m, 3H), 3.95-3.82 (m, 1H), 3.19 (dd, *J* = 11.3, 4.8 Hz, 1H), 3.02-2.94 (m, 2H), 2.80-2.50 (m, 3H), 2.31 (dd, *J* = 11.3, 10.1 Hz, 1H), 2.07 (s, 3H), 2.02 (s, 6H), 2.01 (s, 3H), 1.82-1.22 (m, 26H), 1.14-1.00 (m, 1H); $^{13}$C NMR (75 MHz, $CDCl_3$): δ 171.0, 170.5, 170.2, 169.9, 157.0, 74.8, 69.7, 69.6, 61.7, 59.7, 54.3, 53.1, 51.8, 50.8, 42.6, 31.8 (2C),

31.0, 29.8 (3C), 27.4, 27.2, 26.3 (2C), 25.8, 25.1, 21.0 (2C), 20.9, 20.9; MS (Pos.) m/z 612.6 [M + H]$^+$; HRMS, C31H54N3O9 [M + H]$^+$ calc. mass 612.3860, found 612.3868.




**(2R,3R,4R,5S)-2-((butyryloxy)methyl)-1-(6-(N-cyclohexylpivalamido) hexyl)-piperidine-3,4,5-triyl tributyrate 8**. According to the above procedure, 3-(tert-butyl)-1-cyclohexyl-1-(6-((2R,3R,4R,5S)-3,4,5-trihydroxy-2-(hydroxymethyl)piperidin-1-yl)hexyl)urea **6** (100 mg, 0.22 mmol) was treated with butyric anhydride for 2 days to afford compound **8** (118.6 mg, 74%) as a clear oil: $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 5.17-5.03 (m, 2H), 5.03-4.92 (m, 1H), 4.24-4.04 (m, 3H), 3.99-3.84 (m, 1H), 3.19 (dd, $J$ = 11.4, 5.0 Hz, 1H), 3.02-2.93 (m, 2H), 2.81-2.68 (m, 1H), 2.67-2.61 (m, 1H), 2.60-2.48 (m, 1H), 2.36-2.14 (m, 9H), 1.86-1.18 (m, 34H), 1.18-1.00 (m, 1H), 0.99-0.87 (m, 12H); $^{13}$C NMR (75 MHz, $CDCl_3$): δ 173.7, 173.0, 172.9, 172.4, 157.0, 74.5, 69.5, 69.3, 61.9, 59.6, 54.3, 53.2, 51.8, 50.8, 42.7, 36.3 (2C), 36.2 (2C), 31.8 (2C), 31.1, 29.9 (3C), 27.5, 27.3, 26.3 (2C), 25.9, 25.3, 18.5(3C), 18.4, 13.8 (4C); m/z 724.7 [M + H]$^+$; HRMS, C39H70N3O9 [M + H]$^+$ calc. mass 724.5112, found 724.5151.




**(2R,3R,4R,5S)-1-(6-(3-(tert-butyl)-1-cyclohexylureido)-hexyl)-2-((isobutyryloxy)methyl)-piperidine-3,4,5-triyl tris(2-methylpropanoate) 9**. According to the above procedure, 3-(tert-butyl)-1-cyclohexyl-1-(6-((2R,3R,4R,5S)-3,4,5-trihydroxy-2-(hydroxymethyl)piperidin-1-yl)hexyl)urea **6** (100 mg, 0.22 mmol) was treated with isobutyric anhydride for a week to afford compound **9** (104.7 mg, 66%) as a clear oil: $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 5.19-5.06 (m, 2H), 5.04-4.91 (m, 1H), 4.24-4.12 (m, 2H), 4.10-4.02 (m, 1H), 3.98-3.86 (m, 1H), 3.17 (dd, $J$ = 11.4, 5.3 Hz, 1H), 3.02-2.92 (m, 2H), 2.81-2.38 (m, 7H), 2.36-2.26 (m, 1H), 1.84-1.56 (m, 5H), 1.56-1.20 (m, 21H), 1.19-1.06 (m, 25H); $^{13}$C NMR (75 MHz, $CDCl_3$): δ 176.8, 176.1 (2C), 175.4, 156.8, 74.2, 69.2, 68.8, 62.0, 59.4, 54.1, 53.0, 51.6, 50.6, 42.4, 33.9 (4C), 31.6 (2C), 30.8, 29.6

(3C), 27.3, 27.1, 26.0 (2C), 25.6, 25.4, 18.9 (4C), 18.8 (4C); m/z 724.7 $[M + H]^+$; HRMS, C39H70N3O9 $[M + H]^+$ calc. mass 724.5112, found 724.5140.

**Biological Materials and Methods**

ER α-*glucosidase I Purification.* All procedures were performed at 4 °C. Frozen pig liver (150g; Pel-Freez) was thawed, cut up and homogenized with 250ml of 50mM Tris-HCl buffer, pH 7.2, containing 0.2M sucrose, 5μM, leupeptin, 15μM pepstatin A, 0.5mM PMSF, and 1μM 6-aminohexanoic acid. To obtain the microsomal fraction, the resulting homogenate was filtered through cheesecloth, and the filtrate was centrifuged at 15,000xg for 30 min. The supernatant was centrifuged at 150,000xg for 60 min. The resulting pellet was resuspended in 24 ml of 10mM sodium phosphate buffer, pH 6.8, containing 0.5% v/v Triton X-100. The mixture was stirred for 30 min, and then centrifuged at 150,000xg for 60 min. The supernatant was removed and stored at 4 °C for ER α-Glucosidase II purification, while the Triton-extracted pellet was suspended in 50 ml of 0.2M sodium phosphate buffer, pH 6.8, containing 1% Thesit, and stirred for 60 min. The mixture was then centrifuged at 150,000xg for 90 min, and the supernatant, which contains ER α-glucosidase I, was removed and mixed with 2.5 ml of CPDNJ-Affi-Gel for 18 hours. The gel was recovered by low speed centrifugation. After washing the gel on a sintered-glass filter with 75 ml of 0.1M sodium phosphate buffer, pH 7.0, containing 1% Thesit (Buffer A), alpha-glucosidase I was eluted with 2.5 ml Buffer A containing 100mM N-butyldeoxynojirimycin (NB-DNJ). Using the same 2.5 ml of Buffer A with NB-DNJ, the procedure was repeated three times. After a 15 min wait, alpha-glucosidase I was eluted with another 2.5 ml of Buffer A with NB-DNJ. NB-DNJ was removed from the enzyme by dialysis against 4x1 litres of Buffer A containing 0.02% sodium azide at 4 °C. Enzyme-active fractions were collected and stored at 4 °C or -20 °C. Enzyme was further concentrated with spin filters (Millipore Amicon Ultra-0.5, 10K cut-off centrifugal filter devices) as necessary.

*CPDNJ -Affi-gel column synthesis.* 50mg of CP-DNJ (Toronto Research Chemicals, Inc.) was dissolved in 2ml of deionized water. EDAC (Bio-Rad) was dissolved in 1 ml of dH2O to make a final concentration of 1 M. 5ml of packed Affi-gel was prepared by centrifugation. The supernatant was removed and 1ml of $dH_2O$ and 2ml of CP-DNJ were added to the beads. The solution was titrated to pH 4.8 with 200 mM HCl and 9 ml of $dH_2O$ were added. 1ml of EDAC made before was added and the solution was mixed for 30 min while the pH was kept constant at 4.8 with additional 200mM HCl as necessary. After 30 min, the solution was left to mix at 4 °C overnight. The Affi-gel beads were then washed on a sintered glass with 25ml of alternate cycles of 50mM NaOAc, pH 4.5, with 0.5M NaCl and 50mM Tris-HCl, pH 8.0, with 0.5M NaCl for three times. The beads were finally equilibrated in 0.1M sodium phosphate buffer, pH 7.0, with 1% Thesit and 0.02% sodium azide. The beads were retrieved and stored in the equilibrating buffer until use.

ER α-*glucosidase II Purification.* Using a sintered glass, Con A Sepharose 4B beads (GE Healthcare) were washed with about 100 ml of 20 mM Tris-HCl, pH 7.2, with 0.1 M NaCl, 1mM $MnCl_2$, 1 mM $CaCl_2$ and 0.5% Triton X-100. About 3 ml of Con A Sepharose 4B beads were retrieved and mixed with the Triton X-100 supernatant from the Glucosidase I Purification procedure for 1 hour at 4 °C. The beads were recovered by centrifuging 1000 rpm for 1 min and washed in 20 mM Tris-HCl, pH 7.2, with 0.1M NaCl and 1% w/v octylglucoside. After wash,

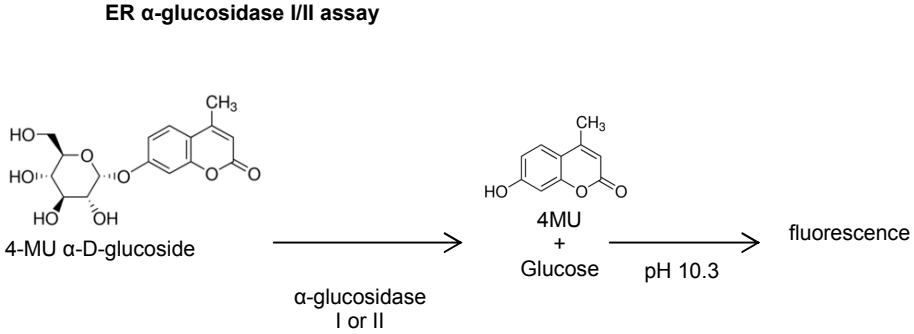
the beads were left to mix overnight at 4 degrees Celsius in 20mM Tris-HCl, pH 7.2, with 0.1 M NaCl, 1% w/v octylglucoside and 0.5 M methyl mannoside. After low speed centrifuge, the supernatant, which contains the eluted ER-glucosidase II, was dialyzed overnight at 4 °C in 20 mM Tris-HCl, pH 7.2, with 0.1M NaCl and 1% w/v octylglucoside.

**Enzymatic assays**

α-*Glucosidase I Assay*. Assay was performed in 96-well Costar flat-bottomed black plate. 10 µl of 4-methylumbelliferyl-a-D-glucoside (Sigma Aldrich) at a concentration of 1.0 mM and 5 µl of sodium phosphate buffer, pH 6.8, with or without compound at 1% DMSO was added to 10 µl of concentrated enzyme, making a total reaction volume of 25 µl. Blanks were made up of 10 µl of the substrate and 15 µl of sodium phosphate buffer, pH 6.8, with or without compound. The reaction was incubated for 90 min at 37 °C. 200 µl of 0.5M glycine, pH 10.3, was added to each reaction, and fluorescence was detected by TECAN plate reader, excitation 350nm, emission 460nm. Background was subtracted for the final values.
α-*glucosidase II Assay.* Assay was performed in 96-well Costar flat-bottomed black plate. 10 µl of 4-methylumbelliferyl-α-D-glucoside (Sigma Aldrich) at a concentration of 0.5 mM and 5 µl of sodium phosphate buffer, pH 6.8, with or without compound at 1% DMSO was added to 10 µl of concentrated enzyme, making a total reaction volume of 20 µl. Blanks were made up of 10 µl of the substrate and 10 µl of sodium phosphate buffer, pH 6.8, with or without compound. The reaction was incubated for 60 min at 37 °C. 200 µl of 0.5M glycine, pH 10.3, was added to each reaction, and fluorescence was detected by TECAN plate reader, excitation 350nm, emission 460nm. Background was subtracted for the final values.

The functional purity of the α-glucosidase preparations was carried out by using alternative 4-methylumbelliferyl glycosides that can be hydrolyzed by β-glucosidase, α or β-mannosidase, α or β-galactosidase.

**Enzymatic assays**




α-*Glucosidase I Assay*. Assay was performed in 96-well Costar flat-bottomed black plate. 10 µl of 4-methylumbelliferyl-a-D-glucoside (Sigma Aldrich) at a concentration of 1.0 mM and 5 µl of sodium phosphate buffer, pH 6.8, with or without compound at 1% DMSO was added to 10 µl of concentrated enzyme, making a total reaction volume of 25 µl. Blanks were made up of 10 µl of

the substrate and 15 µl of sodium phosphate buffer, pH 6.8, with or without compound. The reaction was incubated for 90 min at 37 °C. 200 µl of 0.5M glycine, pH 10.3, was added to each reaction, and fluorescence was detected by TECAN plate reader, excitation 350nm, emission 460nm. Background was subtracted for the final values. α-*glucosidase II Assay.* Assay was performed in 96-well Costar flat-bottomed black plate. 10 µl of 4-methylumbelliferyl-α-D-glucoside (Sigma Aldrich) at a concentration of 0.5 mM and 5 µl of sodium phosphate buffer, pH 6.8, with or without compound at 1% DMSO was added to 10 µl of concentrated enzyme, making a total reaction volume of 20 µl. Blanks were made up of 10 µl of the substrate and 10 µl of sodium phosphate buffer, pH 6.8, with or without compound. The reaction was incubated for 60 min at 37 °C. 200 µl of 0.5M glycine, pH 10.3, was added to each reaction, and fluorescence was detected by TECAN plate reader, excitation 350nm, emission 460nm. Background was subtracted for the final values.

The functional purity of the α-glucosidase preparations was carried out by using alternative 4-methylumbelliferyl glycosides that can be hydrolyzed by β-glucosidase, α or β-mannosidase, α or β-galactosidase.

**The Stability of the prodrugs and the Conversion to Their Parent Compound in Simulated Gastric Fluids**

The working solutions of test compounds and control compound erythromycin were prepared in DMSO at the concentration of 500 µM. 2 µL of the working solution and 198 µL of simulated gastric fluids were added into centrifuge tubes to achieve a final concentration of 5 µM, and incubated at 37°C water bath with shaking at approximately 60 rpm. One of the centrifuge tubes was taken at designated time points including 0, 30, 60, 90 and 120 minutes. The assay was performed in duplicate. The reaction was stopped by adding 5 volumes of cold quench solution (acetonitrile containing internal standards (**IS, 100 nM Alprazolam**, 500 nM Labetalol and 2 µM Ketoprofen)) to the spiked simulated gastric fluids samples at the appointed time points. Another time 0 samples of parent compound were also prepared, and they were used to calculate the conversion percentages of prodrugs to parent compound (used as T0-2). Samples were vortexed for 2 minutes and centrifuged at 20,000 g for 15 minutes at 4°C to precipitate protein. And then 50 µL of the supernatant was transferred to a new 96-well plate with 100 µL quench solution and 100 µL water for LC-MS/MS analysis.

**Data Analysis**: All calculations were carried out using Microsoft Excel. Peak area ratios were determined from extracted ion chromatograms. Percent compounds remaining at each time point were calculated by the following equation:

**Remaining Percentage $_t$ min (%)** = Peak Area Ratio t min/Peak Area Ratio 0 min x 100

where Peak Area Ratio t min is peak area ratio of control and test compounds at t min; Peak Area Ratio 0 min is peak area ratio of control and test compounds at zero-time point.

**Conversion Percentage $_T$ min (%)** = Peak Area Ratio T min/Peak Area Ratio T0-2 x 100

where Peak Area Ratio T min is peak area ratio of IHVR-19029 in prodrug samples at T min; Peak Area Ratio T0-2 stands for peak area ratio of T0-2 samples. while T0-2 indicates a solution of parent compound at same concentration. T0-2 is used to mimic 100% transformation from prodrug to parent compound.

Any value of the compounds that was not within the specified limits was rejected and the experiment was repeated.

| Sample Name | **8** Peak Area (counts) | **IS** Peak Area (counts) |
|---|---|---|
| **8**-GS-120min-1 | 2.04E+06 | 2.38E+05 |
| **8**-GS-120min-2 | 2.08E+06 | 2.72E+05 |
| **8**-GS-90min-1 | 2.07E+06 | 2.40E+05 |
| **8**-GS-90min-2 | 2.07E+06 | 2.54E+05 |
| **8**-GS-60min-1 | 2.34E+06 | 2.50E+05 |
| **8**-GS-60min-2 | 2.11E+06 | 2.67E+05 |
| **8**-GS-30min-1 | 2.08E+06 | 2.28E+05 |
| **8**-GS-30min-2 | 2.09E+06 | 2.26E+05 |
| **8**-GS-0min-1 | 2.35E+06 | 2.45E+05 |
| **8**-GS-0min-2 | 2.13E+06 | 2.46E+05 |

**Table 1**. An example of LC-MS-MS peak area measurements for a prodrug (**8**) and internal standard in SGF stability test.

## The Stability of the prodrugs and the Conversion to Their Parent Compound in Simulated Intestinal Fluids

The working solutions of test compounds and control compound chlorambucil were prepared in DMSO at the concentration of 500 μM. 2 μL of the working solution and 198 μL of simulated intestinal fluids were added into centrifuge tubes to achieve a final concentration of 5 μM, and incubated at 37°C water bath with shaking at approximately 60 rpm. One of the centrifuge tubes was taken at designated time points including 0, 1, 2, 3 and 4 hours. The assay was performed in duplicate. The reaction was stopped by adding 5 volumes of cold quench solution (acetonitrile containing internal standards (**IS, 100 nM Alprazolam**, 500 nM Labetalol and 2 μM Ketoprofen)) to the spiked simulated intestinal fluids samples at the appointed time points. Another time 0 samples of parent compound were also prepared, and they were used to calculate the conversion percentages of prodrugs to parent compound (used as T0-2). Samples were vortexed for 2 minutes and centrifuged at 20,000 g for 15 minutes at 4°C to precipitate protein. And then 50 μL of the supernatant was transferred to a new 96-well plate with 100 μL quench solution and 100 μL water for LC-MS/MS analysis.

**Data Analysis**: All calculations were carried out using Microsoft Excel. Peak area ratios were determined from extracted ion chromatograms. Percent compounds remaining at each time point were calculated by the following equation:

**Remaining Percentage $_{t\ hr}$ (%)** = Peak Area Ratio t hr/Peak Area Ratio 0 hr x 100

where Peak Area Ratio t hr is peak area ratio of control and test compounds at t hr; Peak Area Ratio 0 hr is peak area ratio of control and test compounds at zero-time point.

**Conversion Percentage $_{t\ hr}$ (%)** = Peak Area Ratio T hr/Peak Area Ratio T0-2 x 100

where Peak Area Ratio T hr is peak area ratio of IHVR-19029 in prodrug samples at T hr; Peak Area Ratio T0-2 stands for peak area ratio of T0-2 samples.

Any value of the compounds that was not within the specified limits was rejected and the experiment was repeated.

| Sample Name | **8** Peak Area (counts) | **IS** Peak Area (counts) |
|---|---|---|
| **8**-IS-4h-1 | 1.69E+06 | 8.00E+04 |
| **8**-IS-4h-2 | 1.67E+06 | 8.29E+04 |
| **8**-IS-3h-1 | 1.74E+06 | 7.07E+04 |
| **8**-IS-3h-2 | 1.78E+06 | 7.05E+04 |
| **8**-IS-2h-1 | 1.98E+06 | 7.65E+04 |
| **8**-IS-2h-2 | 2.03E+06 | 8.23E+04 |
| **8**-IS-1h-1 | 2.02E+06 | 7.61E+04 |
| **8**-IS-1h-2 | 2.04E+06 | 7.02E+04 |
| **8**-IS-0h-1 | 2.27E+06 | 8.14E+04 |
| **8**-IS-0h-2 | 2.18E+06 | 7.51E+04 |

**Table 2**. An example of LC-MS-MS peak area measurements for a prodrug (**8**) and internal standard in SIF stability test.

## Caco-2 permeability test

Preparation of Caco-2 Cells. 1) 50 μL and 25 mL of cell culture medium were added to each well of the Transwell insert and reservoir, respectively. And then the HTS transwell plates were incubated at 37 °C, 5% CO2 for 1 hour before cell seeding. 2) Caco-2 cells were diluted to 6.86x106 cells/mL with culture medium and 50 μL of cell suspension were dispensed into the filter well of the 96-well HTS Transwell plate. Cells were cultivated for 14-18 days in a cell culture incubator at 37 °C, 5% CO2, 95% relative humidity. Cell culture medium was replaced every other day, beginning no later than 24 hours after initial plating.

Preparation of Stock Solutions. 10 mM stock solutions of test compounds were prepared in DMSO. The stock solutions of positive controls were prepared in DMSO at the concentration of 10 mM. Digoxin and propranolol were used as control compounds in this assay.

**Assessment of Cell Monolayer Integrity**. 1) Medium was removed from the reservoir and each Transwell insert and replaced with prewarmed fresh culture medium. 2) Transepithelial electrical resistance (TEER) across the monolayer was measured using Millicell Epithelial Volt-Ohm measuring system (Millipore, USA). 3) The Plate was returned to the incubator once the measurement was done. The TEER value was calculated according to the following equation:

TEER measurement (ohms) x Area of membrane ($cm^2$) = TEER value ($ohm•cm^2$)

TEER value should be greater than 230 ohm•cm2, which indicates the well-qualified Caco-2 monolayer.

**Assay Procedures**. 1) The Caco-2 plate was removed from the incubator and washed twice with pre-warmed HBSS (10 mM HEPES, pH 7.4), and then incubated at 37 °C for 30 minutes. 2) The stock solutions of control compounds were diluted in DMSO to get 1 mM solutions and then diluted with HBSS (10 mM HEPES, pH 7.4) to get 5 μM working solutions. The stock solutions of the test compounds were diluted in DMSO to get 1 mM solutions and then diluted with HBSS (10 mM HEPES, pH 7.4) containing 5% BSA or not as designated to get 5 μM working solutions. The final concentration of DMSO in the incubation system was 0.5%. 3) To determine the rate of drug transport in the apical to basolateral direction. 75 μL of 5 μM working solution of test compound was added to the Transwell insert (apical compartment) and the wells in the receiver plate (basolateral compartment) were filled with 235 μL of HBSS (10 mM HEPES, pH 7.4) containing 5% BSA or not as designated. 4) To determine the rate of drug transport in the basolateral to apical direction. 235 μL of 5 μM working solution of test compound was to the receiver plate wells (basolateral compartment) and then the Transwell inserts (apical compartment) were filled with 75 μL of HBSS (10 mM HEPES, pH 7.4) containing 5% BSA or not as designated. Time 0 samples were prepared by transferring 50 μL of 5 μM working solution to wells of the 96-deepwell plate, followed by the addition of 200 μL cold acetonitrile or methanol containing appropriate internal standards (IS). 5) The plates were incubated at 37 °C for 2 hours. 6) At the end of the incubation, 50 μL samples from donor sides (apical compartment for Ap→Bl flux, and basolateral compartment for Bl→Ap) and receiver sides (basolateral compartment for Ap→Bl flux, and apical compartment for Bl→Ap) were transferred to wells of a new 96-well plate, followed by the addition of 4 volumes of cold acetonitrile or methanol containing appropriate internal standards (IS). Samples were Vortexed for 5 minutes and then centrifuged at 3,220 g for 40 minutes. An aliquot of 100 μL of the supernatant was mixed with an appropriate volume of ultra-pure water before LC-MS/MS analysis. 7) To determine the Lucifer Yellow leakage after 2-hour transport period, stock solution of Lucifer yellow was prepared in DMSO and diluted with HBSS (10 mM HEPES, pH 7.4) to reach the final concentration of 100 μM. 100 μL of the Lucifer yellow solution was added to each Transwell insert (apical compartment), followed by filling the wells in the receiver plate (basolateral compartment) with 300 μL of HBSS (10 mM HEPES, pH 7.4). The plates were Incubated at 37 °C for 30 mins. 80 μL samples were removed directly from the apical and basolateral wells (using the basolateral access holes) and transferred to wells of new 96 wells plates. The Lucifer Yellow fluorescence (to monitor monolayer integrity) signal was measured in a fluorescence plate reader at 485 nM excitation and 530 nM emission.

**Data Analysis**. The apparent permeability coefficient (Papp), in units of centimeter per second, can be calculated for Caco-2 drug transport assays using the following equation:

$$\textbf{Papp} = (VA \times [drug]_{acceptor})/(Area \times Time \times [drug]_{acceptor})$$

Where VA is the volume (in mL) in the acceptor well, Area is the surface area of the membrane (0.143 cm2 for Transwell-96 Well Permeable Supports), and time is the total transport time in seconds.

The leakage of Lucifer Yellow, in unit of percentage (%), can be calculated using the following equation:

$$\textbf{\%LY leakage} = 100 \times [LY]_{acceptor}/([LY]_{donor}+[LY]_{acceptor})$$

LY leakage of <1% is acceptable to indicate the well-qualified Caco-2 monolayer.

| Compound | Permeability in Caco-2 | | | Recovery (%) | |
|---|---|---|---|---|---|
| | $P_{app\ (A-B)}$ ($10^{-6}$, cm/s) | $P_{app\ (B-A)}$ ($10^{-6}$, cm/s) | Efflux Ratio | AP-BL | BL-AP |
| **6** | < 0.13 | 8.39 | > 64.90 | < 75 | 84 |
| **7** | 0.94 | 0.96 | 1.02 | 9 | 42 |
| **8** | < 0.18 | < 0.04 | > 0.24 | < 55 | < 47 |
| **9** | < 0.11 | 0.03 | > 0.28 | < 47 | 27 |

**Table 3**. Permeability and recovery data of parent compound **6** and prodrug **7**-**9**.

| Compound ID | TEER $_{A-B}$ ($\Omega \cdot cm^2$) | TEER $_{B-A}$ ($\Omega \cdot cm^2$) | LY Leakage $_{A-B}$ (%) | LY Leakage $_{B-A}$ (%) |
|---|---|---|---|---|
| Propranolol | 914 | 812 | 0.13 | 0.42 |
| Digoxin | 976 | 869 | 0.13 | 0.23 |
| **6** | 989 | 1021 | 0.11 | 0.16 |
| **7** | 992 | 994 | 0.13 | 0.13 |
| **8** | 981 | 1081 | 0.12 | 0.11 |
| **9** | 963 | 1022 | 0.11 | 0.13 |

**Table 4**. The Assessment of Caco-2 Cell Monolayer Integrity

**Stability of prodrugs 7-9 and conversion to 6 in human and mouse intestinal S9 fractions**

A master solution in the "Incubation Plate" containing phosphate buffer, ultra-pure $H_2O$, $MgCl_2$ solution and intestinal S9 fraction was made accordingly. The mixture was pre-warmed at 37°C water bath for 5 minutes. 1) Procedure for 0 min Samples. 89 µL of the master solution described in Table 31 was transferred to a new plate containing 10 µL of 10 mM NADPH solution. The final concentration of NADPH was 1 mM. The negative control samples were prepared by replacing NADPH solution with 10 µL of ultra-pure $H_2O$. Then 600 µL of cold quench solution (acetonitrile containing internal standards (IS, 100 nM Alprazolam, 500 nM Labetalol and 2 µM Ketoprofen)) was added. The plates were vortexed for 5 minutes followed by the addition of 1 µL of 200 µM test compounds solution or control compound solution (verapamil). Samples with

NADPH was prepared in duplicate. Negative controls were prepared in singlet. Another time 0 samples of parent compound were also prepared, and they were used to calculate the conversion percentages of prodrugs to parent compound (used as T0-2). The plate was vortexed for 5 minutes and centrifuged at 3,220 g at 4 °C for 40 minutes to precipitate protein. 2) Procedure for Stability Determination. 3 μL of 200 μM test compounds solution or control compound solution (verapamil) was added to the remaining master solution of the plate at the final concentration of 2 μM. Then 30 μL of 10 mM NADPH solution was added and the final concentration of NADPH was 1 mM. The negative control samples were prepared by replacing NADPH solution with 30 μL of ultra-pure $H_2O$. Samples with NADPH was prepared in duplicate. Negative controls were prepared in singlet. 50 μL aliquots of the reaction solution were added into new plates for different time points including 15, 30, 45 and 60 minutes and incubated at 37°C water bath with shaking at 60 rpm. The reaction was stopped by adding 300 μL of cold quench solution (acetonitrile containing internal standards (IS, 100 nM Alprazolam, 500 nM Labetalol and 2 μM Ketoprofen)) at the appointed time points. The plates were vortexed for 5 minutes and centrifuged at 3,220 g at 4 °C for 40 minutes to precipitate protein. 3) Procedure for Sample Analysis. 100 μL of the supernatant was transferred to a new plate. The supernatant was diluted with 100 μL or 200 μL water according to the LC-MS signal response and peak shape, mixed well and analyzed using LC-MS/MS.

Data Analysis: All calculations were carried out using Microsoft Excel. Peak areas were determined from extracted ion chromatograms. The slope value, k, was determined by linear regression of the natural logarithm of the remaining percentage of the parent drug vs. incubation time curve.

The in vitro half-life (in vitro $t_{1/2}$) was determined from the slope value:

$$\textit{In vitro}\ \mathbf{t_{1/2}} = -(0.693/k)$$

Percent compounds remaining (%) and percent conversion (%) at each time point were calculated by the following equations:

$$\textbf{Remaining Percentage}_{\textbf{t min}}\ \textbf{(\%)} = \text{Peak Area Ratio t min/Peak Area Ratio 0 min} \times 100$$

where Peak Area Ratio t min is peak area ratio of control and test compounds at t min; Peak Area Ratio 0 min is peak area ratio of control and test compounds at zero-time point.

$$\textbf{Conversion Percentage}_{\textbf{T min}}\ \textbf{(\%)} = \text{Peak Area Ratio T min/Peak Area Ratio T0-2} \times 100$$

where Peak Area Ratio T min is peak area ratio of IHVR-19029 in prodrug samples at T min; Peak Area Ratio T0-2 stands for peak area ratio of T0-2 samples.

| cmpd | $T_{1/2}$ (min) | | Remaining in IS9 (%, 1hr) | | | | Converted in IS9 (%) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | H | M | | | M | | H | | M | |
| | | | +cofactor | -cofactor | +cofactor | -cofactor | +cofactor | -cofactor | +cofactor | -cofactor |
| **6** | 594 | 154 | 94 | 95 | 76 | 92 | | | | |
| **7** | 12 | 7 | 3 | 8 | 0 | 9 | 13 | 16 | 1 | 4 |
| **8** | 24 | 16 | 32 | 37 | 6 | 23 | 1 | 2 | 4 | 18 |
| **9** | 36 | 18 | 45 | 52 | 8 | 49 | 1 | 1 | 0 | 3 |

**Table 5**. Stability and conversion evaluation of prodrug**7**-**9** and parent **6** in intestinal S9.

**Metabolic Stability of Prodrugs in Pooled Human and Male Mouse Liver Microsomes**

Two separated experiments were performed as follows. a) With Cofactors (NADPH and UDPGA): 10 μL of 20 mg/mL liver microsomes, 40 μL of 10 mM NADPH and 40 μL of 50 mM UDPGA were added to the incubations. The final concentrations of microsomes, NADPH and UDPGA were 0.5 mg/mL, 1 mM and 5 mM, respectively. b) Without Cofactors (NADPH and UDPGA): 10 μL of 20 mg/mL liver microsomes and 80 μL of ultra-pure H2O were added to the incubations. The final concentration of microsomes was 0.5 mg/mL. The reaction was started with the addition of 4 μL of 200 μM control compound or test compound solutions. Verapamil was used as positive control in this study. The final concentration of test compound or control compound was 2 μM. Aliquots of 50 μL were taken from the reaction solution at 0, 15, 30, 45 and 60 min. The reaction was stopped by the addition of 4 volumes of cold acetonitrile with **IS** (100 nM **Alprazolam**, 200 nM Labetalol and 2 μM Ketoprofen). Samples were centrifuged at 3, 220 g for 40 minutes. Aliquot of 90 μL of the supernatant was mixed with 90 μL of ultra-pure H2O and then used for LC-MS/MS analysis.

**Data Analysis:** All calculations were carried out using Microsoft Excel. Peak areas were determined from extracted ion chromatograms. The slope value, k, was determined by linear regression of the natural logarithm of the remaining percentage of the parent drug vs. incubation time curve.

The in vitro half-life (in vitro $t_{1/2}$) was determined from the slope value:

$$\textit{In vitro}\ \mathbf{t_{1/2}} = -(0.693/k)$$

Conversion of the in vitro $t_{1/2}$ (min) into the in vitro intrinsic clearance (in vitro $CL_{int}$, in μL/min/mg protein) was done using the following equation (mean of duplicate determinations):

$$\textbf{In vitro CLint} = (0.693/t_{1/2}) \times (\text{volume of incubation in } \mu L/\text{amount of proteins in mg})$$

Conversion of the in vitro $t_{1/2}$ (min) into the scale-up unbound intrinsic clearance (Scale-up $CL_{int}$, in mL/min/kg) was done using the following equation (mean of duplicate determinations):

$$\textbf{Scale-up CLint} = (0.693/t_{1/2}) \times (\text{volume of incubation in mL/amount of proteins in mg}) \times \text{scaling factor}$$

| Species | Liver Weight (g liver/kg body weight)[a] | Microsomal Concentration (mg/g liver)[b] | Liver blood flow (Q, mL/min/kg)[a] | Scaling Factor |
|---|---|---|---|---|
| Human | 25.7 | 48.8 | 20.7 | 1254.2 |
| Rat | 40.0 | 44.8 | 55.2 | 1792.0 |
| Mouse | 88.0 | 50.0 | 90.0 | 4400.0 |
| Dog | 32.0 | 77.9 | 30.9 | 2492.8 |
| Monkey | 30.0 | 50.0 | 43.6 | 1500.0 |

**Table 6**. Scaling Factors for Intrinsic Clearance Prediction in Liver Microsomes.

a. Iwatsubo et al, Davies and Morris, 1993, 10 (7) pp 1093-1095.
b. Barter et al, 2007, Curr Drug Metab, 8(1), pp 33-45; Iwatsubo et al, 1997, JPET, 283 pp 462-469.

## The Stability of the prodrugs and the Conversion of to Their Parent Compound in Human and Mouse Plasma

Preparation of Working Solutions. 1 mM working solutions of test compounds were prepared in DMSO, 1 mM working solution of control compound propantheline bromide was prepared in acetonitrile, and then 4 µL of working solution was spiked to 796 µL of pre-incubated human or mouse plasma to reach a final concentration of 5 µM. The final concentration of solvent was 0.5%.

Procedure for Stability Determination. 50 µL aliquots of the spiked plasma were added into new tubes for different time points including 15, 30, 45, 60 and 120 minutes and incubated at 37°C water bath with shaking at 60 rpm. The assay was performed in duplicate. The reaction was stopped by adding 300 µL of room temperature quench solution (acetonitrile containing internal standards (IS, 100 nM Alprazolam, 500 nM Labetalol and 2 µM Ketoprofen)) to the spiked plasma samples at the appointed time points. Time 0 samples were prepared by adding 50 µL of the spiked plasma to new tubes containing 300 µL of room temperature quench solution. Vortex for 5 minutes. Samples in plate were centrifuged at 3,220 g for 30 minutes at room temperature to precipitate protein. Another time 0 samples of parent compound were also prepared, and they were used to calculate the conversion percentages of prodrugs to parent compound (used as T0-2). Samples were vortexed for 2 minutes and centrifuged at 20,000 g for 15 minutes at 4°C to precipitate protein. And then 50 µL of the supernatant was transferred to a new 96-well plate with 100 µL quench solution and 100 µL water for LC-MS/MS analysis.

**Data Analysis**. All calculations were carried out using Microsoft Excel. Peak area ratios were determined from extracted ion chromatograms. Percent compounds remaining at each time point were calculated by the following equation:

$$\textbf{Remaining Percentage}_{\textbf{t min}}\textbf{(\%)} = \text{Peak Area Ratio}_{\text{t min}}/\text{Peak Area Ratio}_{\text{0 min}} \times 100$$

where Peak Area Ratio t min is peak area ratio of control and test compounds at t min; Peak Area Ratio 0 min is peak area ratio of control and test compounds at zero-time point.

**Conversion Percentage $_{T\ min}$ (%)** = Peak Area Ratio T min/Peak Area Ratio T0-2 x100

where Peak Area Ratio T min is peak area ratio of IHVR-19029 in prodrug samples at T min; Peak Area Ratio T0-2 stands for peak area ratio of T0-2 samples.

| Time Points | Human | | Mouse | |
|---|---|---|---|---|
| | IHVR-19029 (6) | | IHVR-19029 (6) | |
| | Analyte Peak Area (counts) | IS Peak Area (counts) | Analyte Peak Area (counts) | IS Peak Area (counts) |
| 120min-1 | 1.96E+06 | 8.96E+05 | 1.62E+06 | 8.93E+05 |
| 120min-2 | 1.82E+06 | 9.16E+05 | 1.80E+06 | 9.19E+05 |
| 60min-1 | 1.92E+06 | 8.74E+05 | 2.13E+06 | 9.52E+05 |
| 60min-2 | 1.76E+06 | 9.19E+05 | 1.96E+06 | 1.04E+06 |
| 45min-1 | 1.80E+06 | 8.85E+05 | 1.75E+06 | 9.67E+05 |
| 45min-2 | 1.93E+06 | 9.49E+05 | 1.75E+06 | 9.44E+05 |
| 30min-1 | 1.82E+06 | 9.24E+05 | 1.97E+06 | 1.08E+06 |
| 30min-2 | 1.86E+06 | 9.09E+05 | 1.79E+06 | 9.23E+05 |
| 15min-1 | 1.79E+06 | 8.80E+05 | 1.75E+06 | 9.57E+05 |
| 15min-2 | 1.86E+06 | 9.13E+05 | 1.91E+06 | 1.02E+06 |
| 0min-1 | 2.01E+06 | 9.87E+05 | 1.98E+06 | 1.02E+06 |
| 0min-2 | 1.87E+06 | 1.04E+06 | 1.77E+06 | 8.45E+05 |

**Table 7**. A representative data acquisition in human and mouse plasma stability assay system for IHVR-19029.

**PHARMACOKINETIC STUDY OF IHVR-19029 AND PRODRUGS 7, 8 AND 9 FOLLOWING A SINGLE DOSE ADMINISTRATION TO MALE BALB/C MICE.** Fifteen mice each group and three mice per time point.

| Group | Compound | Dose Route | Dose Level (mg/kg) | Dose Conc. (mg/ml) | Dose Volume (ml/kg) | Blood Collection Time Points |
|---|---|---|---|---|---|---|
| 1 | **6** | iv | 5 | 1 | 5 | 5, 15, 30 min and 1, 1.5, 2, 4, 6, 8, 24 hr |
| 2 | **6** | po | 25 | 2.5 | 10 | 5, 15, 30 min and 1, 1.5, 2, 4, 6, 8, 24 hr |
| 3 | **6** | po | 100 | 10 | 10 | 5, 15, 30 min and 1, 1.5, 2, 4, 6, 8, 24 hr |
| 4 | **7** | iv | 5 | 1 | 5 | 5, 15, 30 min and 1, 1.5, 2, 4, 6, 8, 24 hr |
| 5 | **7** | po | 25 | 2.5 | 10 | 5, 15, 30 min and 1, 1.5, 2, 4, 6, 8, 24 hr |

| | | | | | | |
|---|---|---|---|---|---|---|
| 6 | **7** | po | 100 | 10 | 10 | 5, 15, 30 min and 1, 1.5, 2, 4, 6, 8, 24 hr |
| 7 | **8** | iv | 5 | 1 | 5 | 5, 15, 30 min and 1, 1.5, 2, 4, 6, 8, 24 hr |
| 8 | **8** | po | 25 | 2.5 | 10 | 5, 15, 30 min and 1, 1.5, 2, 4, 6, 8, 24 hr |
| 9 | **8** | po | 100 | 10 | 10 | 5, 15, 30 min and 1, 1.5, 2, 4, 6, 8, 24 hr |
| 10 | **9** | iv | 5 | 1 | 5 | 5, 15, 30 min and 1, 1.5, 2, 4, 6, 8, 24 hr |
| 11 | **9** | po | 25 | 2.5 | 10 | 5, 15, 30 min and 1, 1.5, 2, 4, 6, 8, 24 hr |
| 12 | **9** | po | 100 | 10 | 10 | 5, 15, 30 min and 1, 1.5, 2, 4, 6, 8, 24 hr |

**Table 8**. Plan for the PK study

**Preparation of Dose Formulations**: Group 1, 2, and 3 dose formulations were prepared by mixing the appropriate amount of test article in PBS to achieve the target concentration, and using a vortex mixer to mix the formulation for 30 to 45 seconds. Group 4 through 12 formulations were prepared by mixing the appropriate amount of test article in 50% NMP/50% solutol solution (20% of final volume) using a vortex mixer for 15 to 30 seconds then adding the remaining saline solution in increments with mixing using a vortex mixer in between. The IV dose formulations were sterile filtered using a 0.2 μm Pall Acrodisc® Syringe Filter

Mice received a single IV dose at 5 mg/kg, or a single PO dose at 25 or 100 mg/kg of **6** and prodrugs **7**, **8**, and **9**. Blood was collected from the treated mice at ten time points, including 5, 15 and 30 minutes and 1, 1.5, 2, 4, 6, 8 and 24 hr post-dose for processing to plasma. All samples were analyzed by LC-MS/MS for drug levels.

For intravenous injection, test articles were administered via the bolus tail vein of male rats (BALB/c, Male, 21.6–25.7 g, 11 weeks old, Charles River Laboratories, Raleigh, NC) at a volume of 5 ml/kg over 5-10 sec time period. For oral administration, test articles were given at a volume of 10 ml/kg (Dose volumes were calculated based on the animal's Day 1 body weight); Duration of In-life Phase: 24 hr.

**Method of Collection of Drug Levels in Plasma:** Blood from the retro-orbital sinus of mice under isoflurane anesthesia was collected in tubes containing K3 EDTA, processed to plasma, and stored frozen at ≤–70°C. Maximum ~400 μl whole blood (~150 μl of plasma) per sample.

**Bioanalytical Method**:

**Compound 6 Calibration Standards and QCs**. Calibration standards (1, 5, 10, 25, 50, 100, 1000, and 5000ng/ml and QC samples (2, 500, and 2500 ng/ml) were prepared in blank pooled male BALB/c mouse plasma as follows. A primary stock solution of 1 mg/ml **6** was prepared by adding 1.08 ml of dimethyl sulfoxide to 1.08 mg of **6** to achieve a final concentration of 1.0 mg/ml. This 1 mg/ml solution was then used to prepare various spiking solutions by dilution with dimethyl sulfoxide. One volume of spiking solution was added to 99 volumes of blank male BALB/c mouse plasma to attain the nominal concentrations of standards with a final non-plasma

matrix of 1.0%. These calibration standards and QCs were prepared and stored at -70°C (nominal), thawed, and analyzed in triplicate on each day of study sample analysis.

**Prodrug Calibration Standards and QCs**. For **7** and **8**, post-extracted spiked calibration standards were prepared at 5, 25, 50, 100, 1000, and 5000 ng/ml, and post-extracted spiked QC samples were prepared at 10, 500, and 2500 ng/ml. For **9**, post-extracted spiked calibration standards were prepared at 1, 5, 10, 25, 50, 100, 1000, and 5000 ng/ml, and post-extracted spiked QC samples were prepared at 2, 500, and 2500 ng/ml. All preparations were performed as follows. Primary stock solutions of the prodrugs in DMSO (2 mg/ml) were prepared by adding an appropriate volume of DMSO to pre-weighed prodrug contained in a glass vial, and then these stocks were further diluted to 1 mg/ml by mixing with an equal volume of DMSO. This 1 mg/ml solution was then used to prepare various spiking solutions by dilution with dimethyl sulfoxide. The neat standards of the prodrugs were prepared by mixing 1 volume of spiking solution to 99 volumes of 50% acetonitrile in water with 0.1% formic acid to attain the nominal concentrations of standards and QCs. Note that this 50% acetonitrile in water with 0.1% formic acid also contained 10 µg/ml hexyl nicotinate when preparing **7** post-extracted spiked standards and QCs, or it contained 10 µg/ml decyl nicotinate when preparing **8** and **9** post-extracted spiked standards and QCs. Several replicate 50 µl aliquots of blank mouse plasma were extracted with 1000 µl each of ethyl acetate. The tubes containing blank plasma and ethyl acetate were vortexed 10 min, and then centrifuged 10 min at 18000g to facilitate phase separation. Then 850 µl of the organic layers were transferred to clean tubes, and the solvent was evaporated under vacuum. The dried residues were reconstituted with 100 µl of the neat standard solutions. These post-extracted spiked standards and QCs were prepared fresh in triplicate on each day of study sample analysis.

**Plasma Sample Processing**: **Compound 6 Sample Preparation for Plasma Analysis**. The bioanalytical method for analysis and quantification of **6** in male BALB/c mouse plasma (sample volume 50 µl) entailed the addition of 1000 µl of ethyl acetate to each tube for liquid:liquid extraction. The tubes were vortexed for 10 min, and then centrifuged for 10 min at 18000g to facilitate separation of the organic and aqueous phases. Then 850 µl of the organic layers were transferred to clean tubes, and the solvent was evaporated under vacuum. The dried residues were reconstituted with 100 µl of 50% acetonitrile with 0.1% formic acid. The reconstituted samples were then vortexed for 5 min on a multi-tube vortex mixer at one half speed, clarified by centrifugation (18000 g, 5 min), and then transferred to HPLC vials fitted with glass inserts for LC-MS/MS analysis. Study samples were quantitated using a set of calibration standards prepared in blank matrix that were processed in parallel.

**Prodrug 7 Preparation for Plasma Analysis**. The bioanalytical method for analysis and quantification of **7** in male BALB/c mouse plasma (sample volume 50 µl) entailed the addition of 1000 µl of ethyl acetate to each tube for liquid:liquid extraction. The tubes were vortexed for 10 min, and then centrifuged for 10 min at 18000g to facilitate separation of the organic and aqueous phases. Then 850 µl of the organic layers were transferred to clean tubes, and the solvent was evaporated under vacuum. The dried residues were reconstituted with 100 µl of 50% acetonitrile with 0.1% formic acid containing 10 µg/ml hexyl nicotinate (internal standard for the LC-MS/MS assay). The reconstituted samples were then vortexed for 5 min on a multi-tube vortex mixer at one half speed, clarified by centrifugation (18000 g, 5 min), and then

transferred to HPLC vials fitted with glass inserts for LC-MS/MS analysis. Study samples were quantitated using a set of post-extracted calibration standards prepared in blank matrix that were processed in parallel.

**Prodrug 8 and 9 Sample Preparation for Plasma Analysis**. The bioanalytical method for analysis and quantification of **8** or **9** in male BALB/c mouse plasma (sample volume 50 µl) entailed the addition of 1000 µl of ethyl acetate to each tube for liquid:liquid extraction. The tubes were vortexed for 10 min, and then centrifuged for 10 min at 18000g to facilitate separation of the organic and aqueous phases. Then 850 µl of the organic layers were transferred to clean tubes, and the solvent was evaporated under vacuum. The dried residues were reconstituted with 100 µl of 50% acetonitrile with 0.1% formic acid containing 10 µg/ml decyl nicotinate (internal standard for the LC-MS/MS assay). The reconstituted samples were then vortexed for 5 min on a multi-tube vortex mixer at one half speed, clarified by centrifugation (18000 g, 5 min), and then transferred to HPLC vials fitted with glass inserts for LC-MS/MS analysis. Study samples were quantitated using a set of post-extracted calibration standards prepared in blank matrix that were processed in parallel.

**Pharmacokinetics Analysis**: The plasma drug level data were analyzed using Phoenix® WinNonlin® (version 6.3) software to perform noncompartmental modeling. Only data obtained for **6** plasma concentrations were used to determine pharmacokinetics. The following parameters and constants were determined if the data allow: maximal plasma concentration (Cmax), time to maximum plasma concentration (Tmax), area under the plasma concentration-time curve (AUC), terminal elimination half-life (t1/2). Clearance (Cl) and volume of distribution (V) were determined for Group 1, with iv administration.