Supporting Information

Discovery of a Potent HIV Integrase Inhibitor that Leads to a Prodrug with Significant anti-HIV Activity

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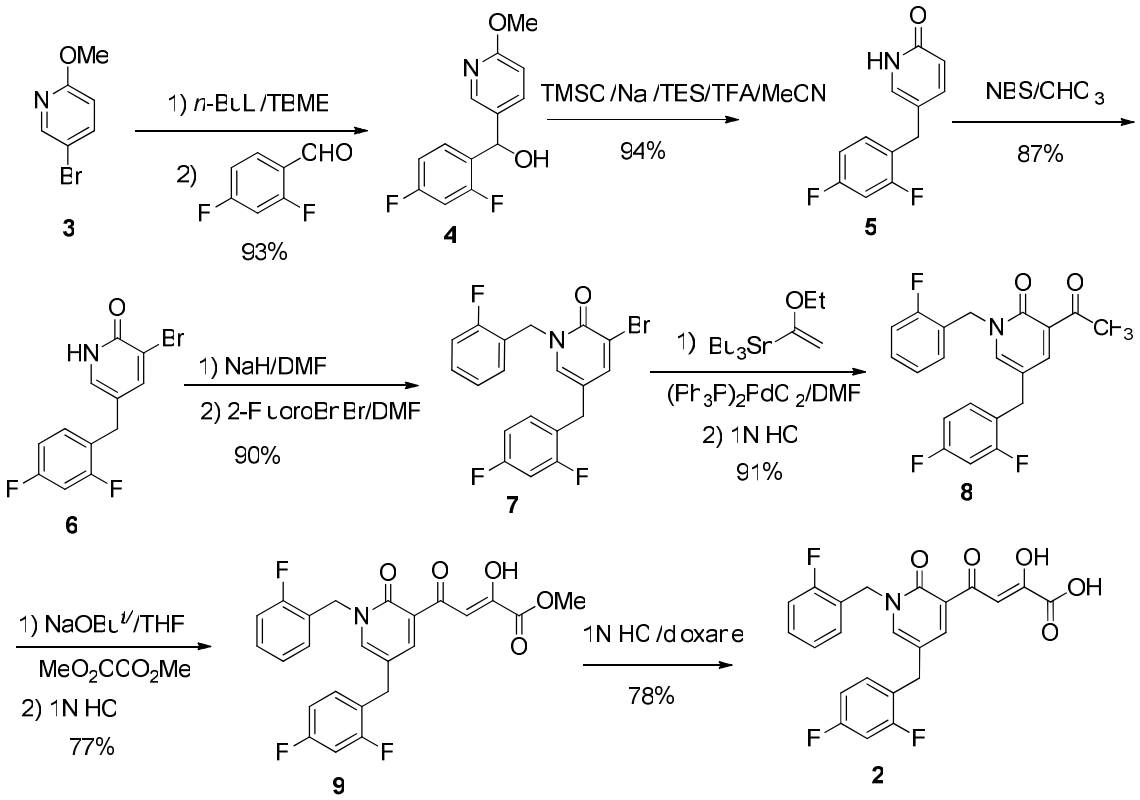
Synthesis of integrase inhibitor 2



(2,4-Difluorophenyl)(6-methoxypyridin-3-yl)methanol (**4**).

To a mixture of 5-bromo-2-methoxypyridine, **3** (2.10 g, 10.6 mmol) in anhydrous *tert*- butyl methyl ether (20 mL) at -32^{o}C and under an argon atmosphere, was added dropwise over 15 min, *n*-BuLi (5.8 mL of a 2 M solution in cyclohexane, 11.7 mmol). After stirring for 1h, 2,4-difluorobenzaldehyde (1.54 g, 10.6 mmol) was added dropwise over 15 min and the reaction mixture was stirred for 1h and then allowed to warm up to 0^{o}C and quenched with saturated NH_4Cl (20 mL). The phases were separated and the aqueous layer was extracted twice with *tert*-butyl methyl ether (20 mL and 10 mL). The combined organic phase was washed with water (50 mL) and dried over anhydrous Na_2SO_4. The solvent was evaporated *in vacuo* and the residue

was purified by flash column chromatography on silica gel (hexanes: ethyl acetate, 85:15). Yield 2.60 g (93%). ^{1}H NMR ($CDCl_3$, 500 MHz): 8.16 (s, 1H), 7.57-7.52 (m, 2H), 6.93-6.76 (m, 2H), 6.72 (d, 1H, *J* = 9.0 Hz), 6.07 (d, 1H, *J* = 3.5 Hz), 3.92 (s, 3H). ^{13}C NMR ($CDCl_3$, 125 MHz): 163.9, 162.4, 159.6, 145.0, 137.2, 131.0, 128.2, 126.5, 111.5, 110.9, 103.9, 67.3, 53.6. HRMS: calcd for $C_{13}H_{12}F_2NO_2$ 252.0836 $[M+H]^+$ found 252.0837.

5-(2,4-Difluorobenzyl)pyridin-2(1*H*)-one (**5**).

A mixture of (2,4-difluorophenyl)-(6-methoxypyridin-3-yl)methanol, **4** (2.28 g, 9.1 mmol), NaI (5.45 g, 36.4 mmol), triethylsilane (2.2 mL, 13.6 mmol) in anhydrous CH_3CN (21 mL) was stirred at room temperature. TFA (1.1 mL, 14.3 mmol) was added at a rate that maintained the temperature below 30^{o}C, and then followed with the addition of TMSCl (5.8 mL, 45.5 mmol). The reaction mixture was heated at 70^{o}C for 3h. Upon cooling to 55^{o}C, Na_2SO_3 (0.8 M, 11.4 mL) was added, and the reaction mixture was concentrated in *vacuo* to 20 mL. A solution of Na_2SO_3 (0.15 M, 11.4 mL) was added and the mixture was stirred at room temperature overnight and then treated with K_2CO_3 (1.4 M, 13.6 mL) at 0^{o}C. The crude product was extracted with ethyl acetate (60 mL) and the extract was washed with Na_2SO_3 (0.15 M, 20mL) and water (60 mL) and then dried over anhydrous Na_2SO_4. The solvent was removed and the residue was purified by flash column chromatography on silica gel ($CHCl_3$: CH_3OH, 90:10). Yield 1.89 g (94 %). ^{1}H NMR ($CDCl_3$, 500 MHz): 13.35 (s, 1H), 7.36-6.79 (m, 7H), 6.53 (d, 1H, *J* = 9.2 Hz), 3.71 (s, 2H). ^{13}C NMR ($CDCl_3$, 125 MHz): 164.7, 162.4, 160.4, 143.2, 132.7, 131.2, 122.3, 120.3, 118.4, 111.4, 104.1, 30.3. HRMS: calcd for $C_{12}H_9F_2NO$, 221.0652 $[M+H]^+$ found 221.0648.

5-(2,4-Difluorobenzyl)-3-bromopyridin-2(1*H*)-one (6).

To a solution of 5-(2,4-difluorobenzyl)pyridin-2(1*H*)-one, **5** (1.89 g, 8.5 mmol) in $CHCl_3$ (25 mL) was added NBS (1.67 g, 9.4 mmol) and the mixture was heated under reflux for 2h and then cooled to room temperature. Insoluble succinimide by-product was filtered. The filtrate was washed with water, dried over anhydrous Na_2SO_4 and concentrated. The resulting brown solid was purified by trituration with CH_3OH. Yield 2.23 g (87 %). ^{1}H NMR ($CDCl_3$, 500 MHz): 13.59 (s, 1H), 7.78 (s, 1H), 7.30- 6.84 (m, 4H), 3.74 (s, 2H). ^{13}C NMR ($CDCl_3$, 125 MHz): 163.2, 163.1, 161.8, 161.2, 161.1, 161.0, 159.9, 159.8, 144.9, 132.4, 131.3, 131.2, 131.2, 121.7, 121.6, 121.6, 119.4, 115.5, 111.7, 111.7, 111.6, 111.5, 104.5, 104.3, 104.1, 30.1. HRMS: calcd for $C_{12}H_8BrF_2NO$, 298.9757 $[M+H]$,$^+$ found 298.9759.

3-Bromo-5-(2,4-difluorobenzyl)-1-(2-fluorobenzyl)-pyridin-2(1*H*)-one (7).

To a suspension of 3-bromo-5-(2,4-difluorobenzyl)-pyridin-2(1*H*)-one, **6** (5.58 g, 18.6 mmol) in anhydrous DMF (160 mL) was added NaH (60% suspension in mineral oil, 0.82 g, 20.5 mmol) at room temperature. After stirring for 15 min, *o*-fluorobenzyl bromide (3.86 g, 20.5 mmol) was added and the reaction mixture was stirred for 2h. The solvent was removed and the residue was dissolved in EtOAc (200 mL), washed with water (200 mL), followed by brine (200 mL) and then dried over anhydrous Na_2SO_4. The solvent was removed and the residue was purified by flash column chromatography on silica gel (hexanes: EtOAc, 80:20). Yield 6.83 g (90 %). ^{1}H NMR ($CDCl_3$, 500 MHz): 7.59- 6.83 (m, 9H), 5.18 (s, 2H), 3.69 (s, 2H). ^{13}C NMR ($CDCl_3$, 125 MHz): 163.1, 163.0, 162.1, 161.8, 161.7, 161.5, 161.0, 160.1, 159.9, 159.8, 158.3, 142.7, 135.3, 135.3, 132.0, 132.0, 131.2, 131.1, 131.1, 131.1, 130.3, 124.6, 124.6, 122.6, 122.4, 121.9, 121.9,

121.8, 121.8, 117.5, 117.0, 115.5, 115.3, 111.6, 111.6, 111.5, 111.4, 104.3, 104.1, 103.9, 48.0, 30.2. HRMS: calcd for $C_{19}H_{13}BrF_3NO$, 407.0133 $[M+H]^+$ found 407.0127.

3-Acetyl-5-(2,4-difluorobenzyl)-1-(2-fluorobenzyl)pyridin-2(1*H*)-one (8).

A mixture of 3-bromo-5-(2,4-difluorobenzyl)-1-(2-fluorobenzyl)-pyridin-2(1*H*)-one, **7** (6.61 g, 16.1 mmol), bis(triphenylphosphine)palladium(II) chloride (1.13 g, 1.61 mmol) and ethoxyvinyl(tributyl)tin (11.69 g, 32.3 mmol) in anhydrous DMF (145 mL) was heated at 70°C under an argon atmosphere for 1h. The solvent was removed and the residue was dissolved in EtOAc (145 mL), filtered through a pad of celite and the filtrate was stirred with dil. HCl (1N, 145 mL) for 15 min. The organic phase was washed with water (2 x 145 mL) followed by brine (145 mL) and then dried over anhydrous Na_2SO_4. The solvent was removed and the residue was purified by flash column chromatography on silica gel (hexanes: EtOAc, 80:20). Yield 5.47 g (91%). ^{1}H NMR ($CDCl_3$, 500 MHz): 7.98 (d, 1H, J = 2.6 Hz), 7.49-7.44 (m, 2H), 7.35-7.30 (m, 1H), 7.16-7.07 (m, 3H), 6.85-6.78 (m, 2H), 5.17 (s, 2H), 3.71 (s, 2H), 2.67 (s, 3H). ^{13}C NMR ($CDCl_3$, 125 MHz): 197.6, 162.4, 162.1, 160.5, 160.4, 160.2, 144.4, 141.4, 131.4, 131.2, 130.4, 127.7, 124.6, 122.6, 122.1, 116.9, 115.6, 111.5, 104.1, 47.2, 30.9, 30.5. HRMS: calcd for $C_{21}H_{17}F_3NO_2$, 372.1211 $[M+H]^+$ found 372.1200.

Methyl 4-(5-(2,4-difluorobenzyl)-1-(2-fluorobenzyl)-2-oxo-1,2-dihydropyridin-3-yl)-2-hydroxy-4-oxobut-2-enoate (**9**).

To a stirred solution of sodium *tert*-butoxide (4.49 g, 45.3 mmol) in anhydrous THF (230 mL) was added a solution of dimethyl oxalate (5.41 g, 45.3 mmol) in anhydrous THF (60 mL) under an argon atmosphere and the reaction mixture was stirred for 30 min at room temperature. A solution of 3-acetyl-5-(2,4-difluorobenzyl)-1-(2-fluorobenzyl)pyridin-2(1H)-one **8** (4.21 g, 11.3

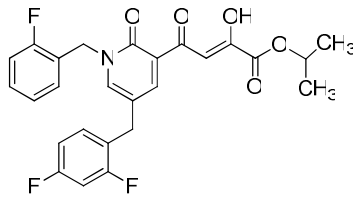
mmol) in THF (60 mL) was added and, after stirring for 3h at room temperature, dil. HCl (1N, 230 mL) was added in one portion at 0-5°C in ice bath. The product was extracted with EtOAc (2 x 230 mL) and the combined organic phase was washed with saturated brine (2 x 230 mL). The solvent was removed affording a brown residue, which was purified by trituration with CH_3OH. Yield 3.99 g (77 %). ^{1}H NMR ($CDCl_3$, 500 MHz): 8.18 (d, 1H, *J* = 3.1 Hz), 7.89 (s, 1H), 7.53 (s, 1H), 7.49-7.46 (s, 1H), 7.35-7.31 (s, 1H), 7.16-7.07 (m, 3H), 6.87-6.80 (m, 2H), 5.20 (s, 2H), 3.90 (s, 3H), 3.75 (s, 2H). ^{13}C NMR ($CDCl_3$, 125 MHz): 185.0, 171.9, 162.8, 162.6, 162.2, 160.6, 160.2, 159.6, 144.8, 142.0, 131.7, 131.2, 130.6, 124.8, 123.6, 122.4, 121.9, 117.7, 115.7, 111.7, 104.3, 101.8, 53.2, 47.6, 30.6. HRMS: calcd for $C_{24}H_{19}F_3NO_5$, 458.1215 $[M+H]^+$, found 458.1225.

4-(5-(2,4-Difluorobenzyl)-1-(2-fluorobenzyl)-2-oxo-1,2-dihydropyridin-3-yl)-2-hydroxy-4-oxobut-2-enoic acid (2).

To a stirred solution of methyl 4-(5-(2,4-difluorobenzyl)-1-(2-fluorobenzyl)-2-oxo-1,2-dihydropyridin-3-yl)-2-hydroxy-4-oxobut-2-enoate, **9** (2.59 g, 5.7 mmol) in 1,4-dioxane (125 mL) was added dil. HCl (1N, 63 mL) and the reaction mixture was heated at 95°C with stirring for 2 h. Solvent was removed and the residual solid was purified by trituration with $CHCl_3$. Yield 1.95 g (78 %).Yellow solid, mp 187- 189°C, UV (CH_3OH) 394 nm (ε 14,917), 320 nm (ε 5,671). ^{1}H NMR ($CDCl_3$-MeOH-d_4, 500 MHz): 8.17 (d, 1H, *J*=3.4 Hz), 7.88 (s, 1H), 7.54 (s, 1H), 7.48-7.45 (m, 1H), 7.36-7.30 (m, 1H), 7.17-7.07 (m, 3H), 6.87-6.81 (m, 2H), 5.19 (s, 2H), 3.76 (s, 2H). ^{13}C NMR (DMSO-d_6, 125 MHz): 184.5, 173.3, 163.2, 161.7, 161.2, 159.8, 158.6, 144.6, 131.9, 130.0, 129.8, 124.5, 123.1, 123.0, 121.5, 116.8, 115.4, 111.6, 103.9, 100.8, 47.3, 29.2. HRMS: calcd $C_{23}H_{17}F_3NO_5$, 444.1059 $[M+H]^+$ found 444.1042.

Synthesis of prodrug 10 from compound 2

Isopropyl 4-(5-(2,4-difluorobenzyl)-1-(2-fluorobenzyl)-2-oxo-1,2-dihydropyridin-3-yl)-2-hydroxy-4-oxobut-2-enoate (10).



10

To a stirred solution of 4-(5-(2,4-difluorobenzyl)-1-(2-fluorobenzyl)-2-oxo-1,2-dihydro-pyridin-3-yl)-2-hydroxy-4-oxo-but-2-enoic acid **2** (0.21 g, 0.467 mmol) in 2-propanol (8 mL) was added conc. H_2SO_4 (1 drop) and the reaction mixture was heated for 6 h at 85ºC. After cooling to room temperature, the mixture was kept at 4^{o}C overnight and then the crude solid product was collected by vacuum filtration and the crude solid product was purified by trituration with 2-propanol to give a pale yellow solid. Yield 0.134 g (59 %), mp. 107-108 ^{o}C. UV (CH_3OH) 397 nm (ε 13,158), 323 nm (ε 5,223). ^{1}H NMR ($CDCl_3$, 500 MHz): 15.27 (s, 1H), 8.15 (d, *J*=2.7 Hz, 1H), 7.84 (s, 1H), 7.51-7.46 (m, 2H), 7.35-7.30 (m, 1H), 7.16-7.07 (m, 3H), 6.86-6.80 (m, 2H), 5.22-5.17 (m, 3H), 3.74 (s, 2H), 1.36 (d, *J*=6.4 Hz, 2H). ^{13}C NMR ($CDCl_3$, 125 MHz): 184.8, 173.9, 163.2, 162.1, 161.9, 161.8, 161.2, 160.2, 159.9, 159.4, 144.4, 141.5, 131.7, 131.1, 130.5, 124.7, 123.7, 122.4, 121.9, 121.8, 117.2, 115.6, 111.6, 104.3, 101.4, 70.5, 47.3, 30.6, 21.7. HRMS: calcd for $C_{26}H_{23}F_3NO_5$, 486.1528 $[M+H]^+$, found 486.1504.

Area % Report

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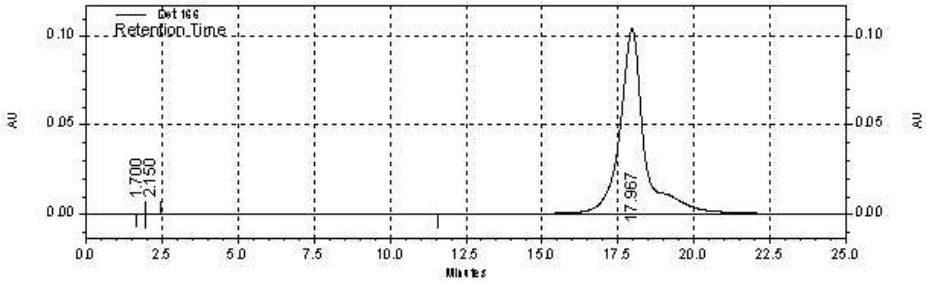

Det 166 Results

Time	Area	Area %	Height	Height %
1.700	487	0.01	56	0.05
2.150	1118	0.02	73	0.07
17.967	6097419	99.97	104528	99.88

Figure S1. Reversed-phase HPLC of **10** supporting its purity of >95 %.

Area % Report

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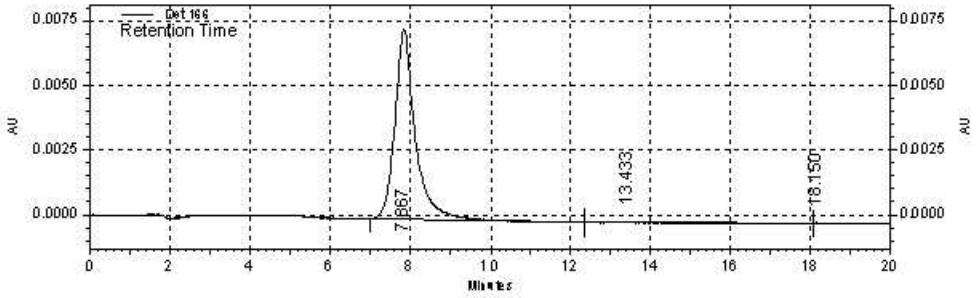

Det 166 Results

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7.867	264343	99.64	7402	99.17
13.433	692	0.26	39	0.52
18.150	265	0.10	23	0.31

Figure S2. Reversed-phase HPLC of compound **2** supporting its purity of >95 %.

MAGI Cell Assays for Anti-HIV Activity

MAGI cells are infected with virus in the presence of test compound. If the virus is able to infect and replicate in the cells it will proceed through reverse transcription and integration and begin transcription from the integrated provirus. One of the first virus proteins produced is HIV-1 Tat, which transactivates the HIV-1 LTR promoter driving expression of β-galactosidase from an LTR-β-galactosidase reporter gene construct engineered into the cells. As a result, infected cells begin to overproduce the β-galactosidase enzyme. Forty-eight hours (single-cycle assay) or 6-days (multi-cycle assay) post infection, the cells are lysed and β-galactosidase enzyme activity is measured using a chemiluminescence detection method (Perkin Elmer Applied Biosystems). Compound toxicity is monitored on replicate plates using MTS dye reduction (CellTiter 96® Reagent, Promega, Madison, WI).

General Method for Cytochrome P-450 Isozyme Assays

Incubation mixtures contained potassium phosphate buffer (100 mM, pH 7.4), $MgCl_2$ (5 mM), the requisite substrate dissolved in an appropriate solvent, compound **2** (range of concentrations) and pooled human liver microsomes. The reaction mixture (final volume 100 μL) was pre-incubated for 3 min at 37 ^{o}C before initiation of the reaction by the addition of NADPH (final concentration 2 mM). Each reaction was terminated by adding ice-cold acetonitrile (100 μL). In each incubation mixture, the organic solvent concentration was kept at or below 1.5% by volume. Proteins were precipitated from the samples by centrifugation at 5000g for 10 min at room temperature in a microcentrifuge tube. The supernatant was analyzed by HPLC-UV (and HRMS where necessary) for detection of substrates and metabolites. Reversed-phase HPLC analyses were performed on a Beckman Coulter Gold 127 solvent system with a Gold 166 UV

analytical detector. The data were collected and processed using a Gold software package. HPLC columns, conditions and elution details are given below.

Table S1. HPLC Assay conditions for CYP 450 isozyme inhibition and related studies

Compounds	Conditions	Description
Testosterone and 6β-hydroxytestosterone	*Mobile Phase Elution*	Gradient of A and B. Flow Rate: 1.2.mL/min A: 0.01 M Potassium phosphate buffer pH 4.5 B: Acetonitrile, 0-3 min, 25 % B, 3- 7 min, 57 % B, 7-14 min, 57 % B 14- 18 min, 100 % B, 18-23 min, 100 % B
	UV Detection	254 nm
	Retention Time	14.48 min and 10.25 min respectively
	Column	Discovery® C18 (5 µm, 250 mm x 4.6 mm, 180 Å)
Triazolam and 4-hydroxytriazolam	*Mobile Phase Elution*	Gradient of A and B. Flow Rate: 1.0 mL/min A: 0.01 M Potassium phosphate buffer pH 3.0 B: Acetonitrile 0-3 min, 30 % B, 3- 7 min, 48% B, 7-9 min, 48 % B 9-11 min, 100 % B, 11- 16 min, 100 % B
	UV Detection	260 nm
	Retention Time	8.9 and 6.2 min, respectively
	Column	Nova-Pak C18 (4 µm, 150 mm x 3.9 mm, 60 Å)
Dextromethorphan and Dextrorphan	*Mobile Phase Elution*	Gradient of A and B. Flow Rate: 1.0 mL/min A: 0.01 M Potassium phosphate buffer pH 3.0 B: 1:1 acetonitrile: methanol (v/v) 0-3 min, 28 % B, 3- 8 min, 60 % B, 8-10 min, 60 % B 10-12 min, 100 % B, 12- 16 min, 100 % B
	UV Detection	278 nm
	Retention Time	10.2 min and 4.9 min, respectively.
	Column	Nova-Pak C18 (4 µm, 150 mm x 3.9 mm, 60 Å)
Amodiaquine and N-desethylamodiaquine	***Mobile Phase Elution***	**Gradient of A and B. Flow Rate: 1.0 mL/min A: 0.01 M Potassium phosphate buffer pH 3.0 B: acetonitrile: methanol (250:200 v/v) 0-5 min, 18 % B, 5- 7 min, 32 % B, 7-10 min, 100 % B 10-18 min, 100 % B**
	UV Detection	**342 nm**
	Retention Time	**6.5 min and 5.1 min, respectively.**
	Column	**Nova-Pak C18 (4 µm, 150 mm x 3.9 mm, 60 Å)**

Microsome Stability Assays

The incubation mixture (final volume of 400 µL) contained liver microsomes (microsomal protein, 0.5 mg/mL), compound 2 **(200 µM in DMSO), G-6-P-DH (0.5 U/mL), and G-6-P (5 mM) in potassium phosphate buffer (100 mM, pH 7.4) containing $MgCl_2$ (5 mM). DMSO used was kept below 0.5% by volume in the final incubation mixture. The reaction mixture was pre-incubated for 3 min at 37 ºC before the addition of NADPH (final concentration 2 mM) and then incubated further at 37 ºC with continued sampling as described. An aliquot (60 µL) of the incubation mixture was taken in a microcentrifuge tube and quenched through the addition of ice cold acetonitrile (120 µL). Sampling intervals of 0, 30, 120 and 180 min were used. The proteins were removed by centrifugation at 5,000g for 5 min at room temperature then the supernatant was analyzed by HPLC-UV (HRMS where needed) to monitor the compound and its metabolite over time. Reversed-phase HPLC analyses were performed on a Beckman Coulter Gold 127 solvent system with a Gold 166 UV analytical detector. The data were collected and processed using a Gold software package. The elution process involved: analytical column Delta Pak C18 (15 µm, 300 mm x 3.9 mm, 100 Å) with a mobile phase of (A)10 mM potassium phosphate buffer (pH 6.5) and (B) acetonitrile, which were used as follows: 0-2 min, 30 % B, 2- 8 min, 30-60 % B, 8- 18 min, 60 % B. The flow rate was kept at 1.5 mL/min, with UV detection at 360 nm. Retention times of compound** 2 **and its minor cleavage product,** 8 **[<5%)] were 7.8 and 13.4 min, respectively, under these HPLC conditions.**

UGT-catalyzed Glucuronidation Studies

Final incubation mixtures (final volume of 500 µL) contained pooled human liver microsomes (microsomal proteins, 1.0 mg/mL), compound 2 or raltegravir (50 µM in DMSO), UDPGA (4 mM), alamethicin (0.024 mg/mg protein), D-saccharic acid 1,4-lactone (10 mM) in potassium phosphate buffer (100 mM, pH 7.4) containing $MgCl_2$ (5 mM). Initially, the mixture of liver microsomes and alamethicin in potassium phosphate buffer containing $MgCl_2$ was kept in ice (0°C) for 15 min. D-saccharic acid-1,4-lactone and the test compound was then added. This mixture was preincubated at 37 °C for 3 minutes and UDPGA (4 mM) was then added to initiate the reaction. The DMSO used was held below 1.0 % by volume in the final incubation mixtures. An aliquot (60 µL) of the incubation mixture was removed at intervals of 0, 30, 60, and 120 min and placed in a microcentrifuge tube and the reaction terminated by the addition of ice cold acetonitrile (60 µL). The precipitated proteins were removed by centrifugation at 5,000g for 5 min at room temperature before separation, analysis and detection of the test compound and its glucuronide in the supernatant by HPLC/UV and HRMS (where needed). Reversed-phase HPLC analyses for glucuronidation studies for compound 2 were performed on a Beckman Coulter Gold 127 solvent system with a Gold 166 UV analytical detector. The data were collected and processed using a Gold software package. The elution process involved: analytical column Delta Pak C18 (15 micron, 300 mm x 3.9 mm, 100 Å) with a mobile phase of (A)10 mM potassium phosphate buffer (pH 6.5) and (B) acetonitrile, which were used as follows: 0-3 min, 12% B, 3-7 min, 12-38% B, 7-9 min, 38% B, 9-12 min, 38-65% B, 12-17 min, 65% B. The flow rate was kept at 1.5 mL/min, with UV detection at 350 nm. Reversed-phase HPLC analyses for the glucuronidation of raltegravir were performed on a Beckman Coulter Gold 127 solvent system. The data were collected and processed using a

Gold software package. The elution process involved: analytical column Ultrasphere® dp (5 µm, 150 mm x 4.6 mm, (Beckman)) with a mobile phase of (A) 10 mM monobasic potassium phosphate buffer pH 5.2 and (B) acetonitrile (0.2 % formic acid), which were used as follows: 0-2 min, 10 % B, 2- 40 min, 10-50 % B, 40- 42 min, 10 % B, 42- 49 min 10% B. The flow rate was kept at 1.0 mL/min, with UV detection at 300 nm. HPLC retention times: raltegravir, 30.6 min, its glucuronide 16.7 min; compound 2, 7.8 min, its glucuronide ~ 4 min (not present).