

## Supplementary Tables and Figures

**Supplementary Table 1.** HPLC and mass spectrometry settings of the Orbitrap high resolution mass spectrometry system and the QTRAP 5500 LC-MS/MS system

**Supplementary Table 2.** Radioactive recovery in urine and feces after subcutaneous administration of  $^{14}\text{C}$ -guadecitabine from 0-48h and up to the last point of collection

**Supplementary Table 3.** Summary of pharmacokinetic parameters of guadecitabine and  $\beta$ -decitabine in plasma on Day 1 and Day 5 after subcutaneous administration of guadecitabine at a dose of 45 mg/m<sup>2</sup>

**Supplementary Table 4.** Proposed metabolite structures and fragmentation patterns of identified metabolites in urine using Orbitrap accurate mass analysis

**Supplementary Table 5.** Cumulative recovery of guadecitabine metabolites in urine (mean  $\pm$  SD) from 0-24h and 0-48h, based on total radioactivity measurements following administration of subcutaneous  $^{14}\text{C}$ -guadecitabine at a dose of 45 mg/m<sup>2</sup> (n=5)

**Supplementary Figure 1.** Multiple reaction monitoring chromatograms of a patient urine sample collected approximately **(A)** 4 h, and **(B)** 48 h after administration of subcutaneous guadecitabine 45 mg/m<sup>2</sup> monitoring **(A, B)** decitabine ( ———  $m/z$  229.1  $\rightarrow$  113.1) and **(C, D)** decitabine-IS ( - - - - -  $m/z$  235.1  $\rightarrow$  119.1) mass transitions.

Supplementary Table 1.

<b>Pump</b>	<b>LC-10AD (Shimadzu)</b>		<b>Acquity I Class (Waters)</b>		
<b>Flow rate</b>	1.0 mL/min		1.0 mL/min		
<b>Analytical column</b>	Luna NH2 (250 x 4.6 mm, 5 µm)		Luna NH2 (250 x 4.6 mm, 5 µm)		
<b>Column oven</b>	35 °C		Acquity I Class (Waters)		
<b>Run time</b>	60 min		60 min		
<b>Mobile phase A</b>	IPA: 1M ammonium formate, pH 3.0: ACN (12:0.24:88, v/v/v)		IPA: 1M ammonium formate, pH 3.0: ACN (12:0.24:88, v/v/v)		
<b>Mobile phase B</b>	IPA: 1M ammonium formate, pH 3.0: ACN (49:1:50, v/v/v)		IPA: 1M ammonium formate, pH 3.0: ACN (49:1:50, v/v/v)		
<b>Gradient composition</b>	Mobile phase B: 20% (0-3 min), 20% → 100% (3-28 min), 100% (28-55 min), 100% → 20% (55-55.1 min), 20% (55.1-60 min)		Mobile phase B: 20% (0-3 min), 20% → 100% (3-28 min), 100% (28-55 min), 100% → 20% (55-55.1 min), 20% (55.1-60 min)		
<b>Autosampler</b>	<b>SIL HTc (Shimadzu)</b>		<b>Acquity I Class (Waters)</b>		
<b>Tray temperature</b>	15 °C		5 ± 3 °C		
<b>Injection volume</b>	50 µL		10 µL		
<b>Splitter</b>	<b>Accurate (LC packings)</b>		<b>Accurate (LC packings)</b>		
<b>Split ratio (collector:MS)</b>	3:1 (v/v)		3:1 (v/v)		
<b>Mass spectrometer</b>	<b>LTQ Orbitrap (Thermo)</b>		<b>QTRAP 5500 (AB Sciex)</b>		
<b>Ionization/interface</b>	ESI, positive ionization mode	ESI, negative ionization mode	<b>Ionization/interface</b>	ESI, positive ionization mode	ESI, negative ionization mode
<b>Scan range (m/z)</b>	50-1000	50-1000	<b>Monitored transitions (m/z)</b>	558.1 → 446.0 (SGI-110), 229.1 → 113.0 (DEC), 219 → 103.0 (M5)	128.0 → 85.0 (M1), 244.1 → 85.0 (M2), 246.1 → 129.0 (M3), 218.0 → 102.0 (M4)
<b>Monitored transitions (m/z)</b>	NA	NA	<b>Ionspray voltage (V)</b>	5500	-4500
<b>Sheath gas flow (au)</b>	25	25	<b>Ion source gas 1 (au)</b>	50	50
<b>Aux gas flow (au)</b>	5	5	<b>Ion source gas 2 (au)</b>	50	50
<b>Sweep gas flow (au)</b>	2	2	<b>Curtain gas (au)</b>	30	30
<b>Spray voltage (kV)</b>	3.5	5	<b>Collision gas (au)</b>	8	8
<b>Normalized collision energy (V)</b>	35	40	<b>Temperature (°C)</b>	400	400
<b>Capillary temperature (°C)</b>	300	350	<b>Dwell time (ms)</b>	30	30
<b>Capillary voltage (V)</b>	35	-22	<b>Collision energy (V)</b>	20	-20
<b>Tube lens (V)</b>	52	-43	<b>Collision exit potential (V)</b>	10	-10
			<b>Declustering potential (V)</b>	125	-125

**Supplementary Table 2.**

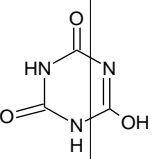
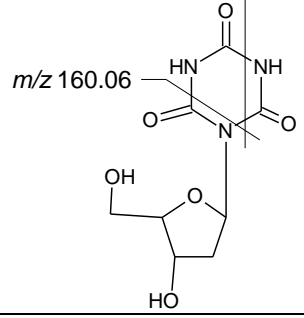
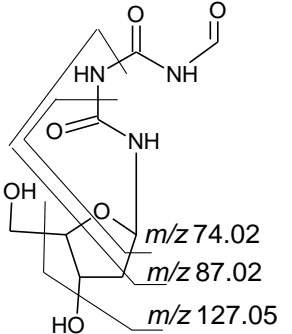
<b>Patient (#)</b>	<b>0-48 h</b>			<b>Up to the last point of collection</b>			
	<b>Urine (%)</b>	<b>Feces (%)</b>	<b>Total (%)</b>	<b>Urine (%)</b>	<b>Feces (%)</b>	<b>Total (%)</b>	<b>Collection period (h)</b>
<b>1</b>	72.3	0.0	72.3	77.4	0.3	77.6	144
<b>2</b>	91.3	0.0	91.3	95.5	0.4	95.8	144
<b>3</b>	82.1	0.2	82.4	86.6	0.5	87.1	144
<b>4</b>	95.6	0.2	95.7	98.2	0.2	98.4	72
<b>5</b>	92.2	0.2	92.4	93.2	0.4	93.6	72
<b>Mean</b>	86.7	0.1	86.8	90.2	0.4	90.5	NA
<b>SD</b>	8.5	0.1	8.5	7.5	0.1	7.4	NA

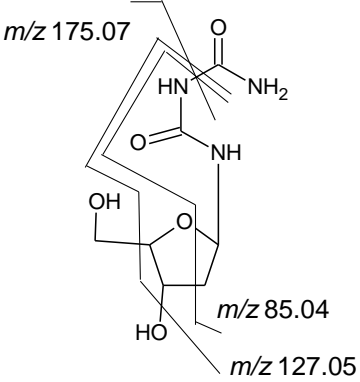
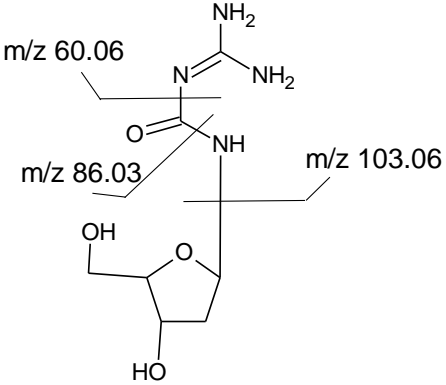
Supplementary Table 3.

Parameter		Guadecitabine Day 1 (n=5)	Guadecitabine Day 5 (n=5)	β-decitabine Day 1 (n=5)	β-decitabine Day 5 (n=5)
<b>C<sub>max</sub> (ng/mL)</b>	Mean	108	165	42.9	51.4
	CV (%)	43.6	46.1	35.1	28.4
<b>T<sub>max</sub> (h)</b>	Mean	1.85	0.90	2.05	1.80
	CV (%)	75.0	24.8	61.2	31.7
<b>AUC<sub>0-last</sub> (ng·h/mL)</b>	Mean	245	269	130	134
	CV (%)	22.0	28.7	31.7	22.0
<b>AUC<sub>0-inf</sub> (ng·h/mL)</b>	Mean	249	275	135	136
	CV (%)	22.0	26.4	33.0	21.9
<b>t<sub>1/2</sub> (h)</b>	Mean	0.78	0.67	1.12	0.95
	CV (%)	37.8	36.8	30.4	7.39
<b>k<sub>e</sub> (h<sup>-1</sup>)</b>	Mean	1.04	1.14	0.68	0.73
	CV (%)	48.3	35.7	34.2	7.91
<b>V<sub>z</sub>/F (L/m<sup>2</sup>)</b>	Mean	204	172	NA	NA
	CV (%)	31.8	49.7	NA	NA
<b>CL/F (L/h/m<sup>2</sup>)</b>	Mean	189	172	NA	NA
	CV (%)	26.6	23.8	NA	NA

NA Not applicable  
*AUC<sub>0-inf</sub>* Area under the plasma concentration-time curve from time 0 to infinity, *AUC<sub>0-last</sub>* Area under the plasma concentration-time curve from time 0 to the last quantifiable concentration, *CL/F* Apparent oral clearance, *C<sub>max</sub>* maximum observed plasma concentration, *CV* Coefficient of variation, *T<sub>max</sub>* time to reach maximum observed plasma concentration, *t<sub>1/2</sub>* terminal half-life, *V<sub>z</sub>/F* Apparent volume of distribution during terminal phase

Supplementary Table 4.

Metabolite	Proposed structure and fragmentation	Retention time (min)	MS	Theoretical monoisotopic ion mass (Da)	Measured monoisotopic ion mass (Da)	$\Delta$ ppm
<b>M1</b>	<p><i>m/z</i> 85.00</p> 	14-16	MS	128.01016	128.01019	0.234356
			MS <sup>2</sup>	85.00435	85.00456	2.470462
<b>M2</b>	<p><i>m/z</i> 201.05</p> <p><i>m/z</i> 160.06</p> 	10.5-12.5	MS	244.05751	244.05733	-0.737531
			MS <sup>2</sup>	201.05169	201.05139	-1.492154
			MS <sup>2</sup>	160.06153	160.06157	0.249904
<b>M3</b>	 <p><i>m/z</i> 74.02</p> <p><i>m/z</i> 87.02</p> <p><i>m/z</i> 127.05</p>	14-16	MS	246.07316	246.07290	-1.056596
			MS <sup>2</sup>	127.05130	127.05122	-0.629667
			MS <sup>2</sup>	87.02000	87.02008	0.919329
			MS <sup>3</sup>	74.02475	74.02493	2.431619

<b>M4</b>		16-20	MS	218.07824	218.07828	0.183420
			MS <sup>2</sup>	175.07243	175.07228	-0.856788
			MS <sup>3</sup>	85.04074	85.04095	2.469405
			MS <sup>3</sup>	127.05130	127.05129	-0.078708
<b>M5</b>		25-35	MS	219.10878	219.10840	-1.734298
			MS <sup>2</sup>	175.07243	175.07228	-0.856788
			MS <sup>3</sup>	85.04074	85.04095	2.469405
			MS <sup>3</sup>	127.05130	127.05129	-0.078708

**Supplementary Table 5.**

<b>Metabolite</b>	<b>% of administered dose</b>	
	<i>0-24h</i>	<i>0-48h</i>
<b>M1</b>	19.6 (3.8)	20.9 (3.7)
<b>M2</b>	14.0 (6.8)	14.3 (6.8)
<b>M3/4</b>	23.0 (4.0)	26.2 (3.3)
<b>M5</b>	11.8 (3.4)	13.9 (3.6)
<b>Total of M1-M5</b>	<b>68.3 (14.1)</b>	<b>75.3 (12.5)</b>
<b>Total radioactivity</b>	<b>77.3 (11.6)</b>	<b>86.7 (9.5)</b>

### Supplementary Figure 1.

