Supplemental Material

Metabolism and pharmacokinetic study of the boron-containing prodrug of belinostat (ZL277), a pan HDAC inhibitor with enhanced bioavailability

Changde Zhang[§], Shanchun Guo[§], Qiu Zhong, Qiang Zhang, Ahamed Hossain, Shilong Zheng*, and Guangdi Wang*

RCMI Cancer Research Center and Department of Chemistry, Xavier University of Louisiana, New Orleans, LA 70125

Contents

Table S1 **Page 2**
Table S2 **Page 2**
Table S3 **Page 3**
Table S4 **Page 3**
Table S5 **Page 3**
Table S6 **Page 4**
Table S7 **Page 4**
Table S8 **Page 4**
Figure S1 **Page 5**
Figure S2 **Page 7**
Sample Preparation Protocols **Page 8**

Table S1. PRM workflow parameters for the Q-Exactive mass spectrometer.

Workflow			
Negative PRM		**Positive PRM**	
General		**General**	
Run time	0-9.3min	Run time	0-9.3min
Polarity	negative	Polarity	positive
In-source-CID	0.0ev	In-source-CID	0.0ev
Default charge	1	Default charge	1
Inclusion	on	Inclusion	on
MS^2		**MS^2**	
Microscans	1	Microscans	1
Resolution	17500	Resolution	17500
AGC Target	2e4	AGC Target	2e4
Max Inject Time (ms)	100ms	Max Inject Time (ms)	100ms
Loop count	1	Loop count	1
Isolation window	4.0 m/z	Isolation window	4.0 m/z
Isolation offset	0.0 m/z	Isolation offset	0.0 m/z
(N)CE/stepped nce	35	(N)CE/stepped nce	35
Spectrum data t	Profile	Spectrum data t	Profile

Table S2. Full MS workflow parameters for the Q-Exactive mass spectrometer.

Workflow	
Properties of Full MS-SIM	
General	
Run time	0-9.3min
Polarity	negative
In-source-CID	0.0ev
Full MS-SIM	
Microscans	1
Resolution	17500
AGC Target	2e6
Max Inject Time (ms)	200ms
Number of scan	1
Scan range	100-1000 m/z
Spectrum data t	Centroid

Table S3. NSI tune parameters for the Q-Exactive mass spectrometer.

Parameters of NSI source	
NSI source	
Sheath gas flow rate	10
Auxgas flow rate	0
Sweep gas flow rate	0
Spray voltage (kv)	1.50
Spray current (μA)	
Capillary temperature (ºC)	250
S-lens RF level	50.0

Table S4. Instrument tune parameters for the TSQ Vantage mass spectrometer.

Parameters of instrument tune setup	
Spray voltage	3200
Vaporizer temperature (ºC)	365
Sheath gas pressure (PSI)	33
Ion sweep gas pressure (PSI)	1.5
Aux gas pressure (PSI)	10
Capillary temperature (ºC)	350
S-lens RF amplitude	51

Table S5. Detect limitations and correlation equations of belinostat, ZL277-B$(OH)_2$-452, and ZL277-OH-424 for plasma samples during validation.

	Belinostat	ZL277-B$(OH)_2$-452	ZL277-OH-424
Limitation of detection	32 pg	23 pg	21 pg
Limitation of quantification	160 pg	115 pg	105 pg
Correlation equations	Y=0.00566X+0.000015 R2=0.9927	Y=0.03849X + 0.00003 R2=0.9909	Y=0.53908X+0.000013 R2=9822

Table S6. Detect limitations and correlation equations of belinostat, ZL277-B(OH)$_2$-452, and ZL277-OH-424 for breast tissue during validation.

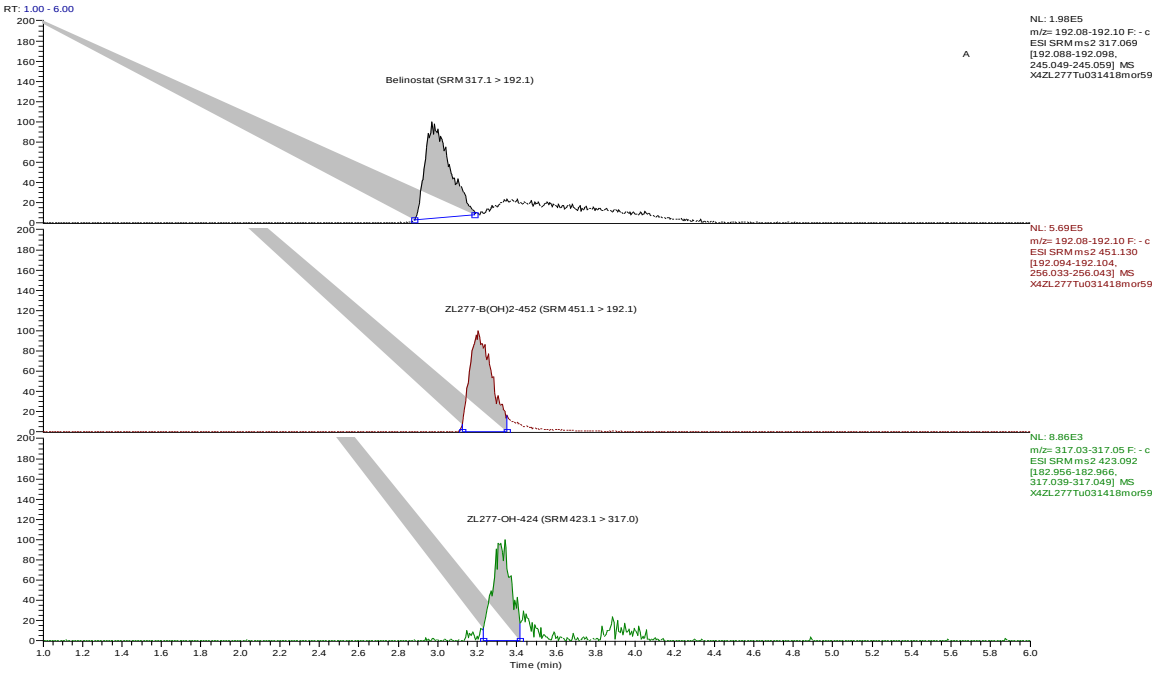
	Belinostat	ZL277-B(OH)$_2$-452	ZL277-OH-424
Limitation of detection	32 pg	23 pg	21 pg
Limitation of quantification	160 pg	115 pg	105 pg
Correlation equations	Y=0.00561X+0.00010 R2=0.9927	Y=0.03632X + 0.00017 R2=0.9702	Y=0.58106X+0.00015 R2=9935

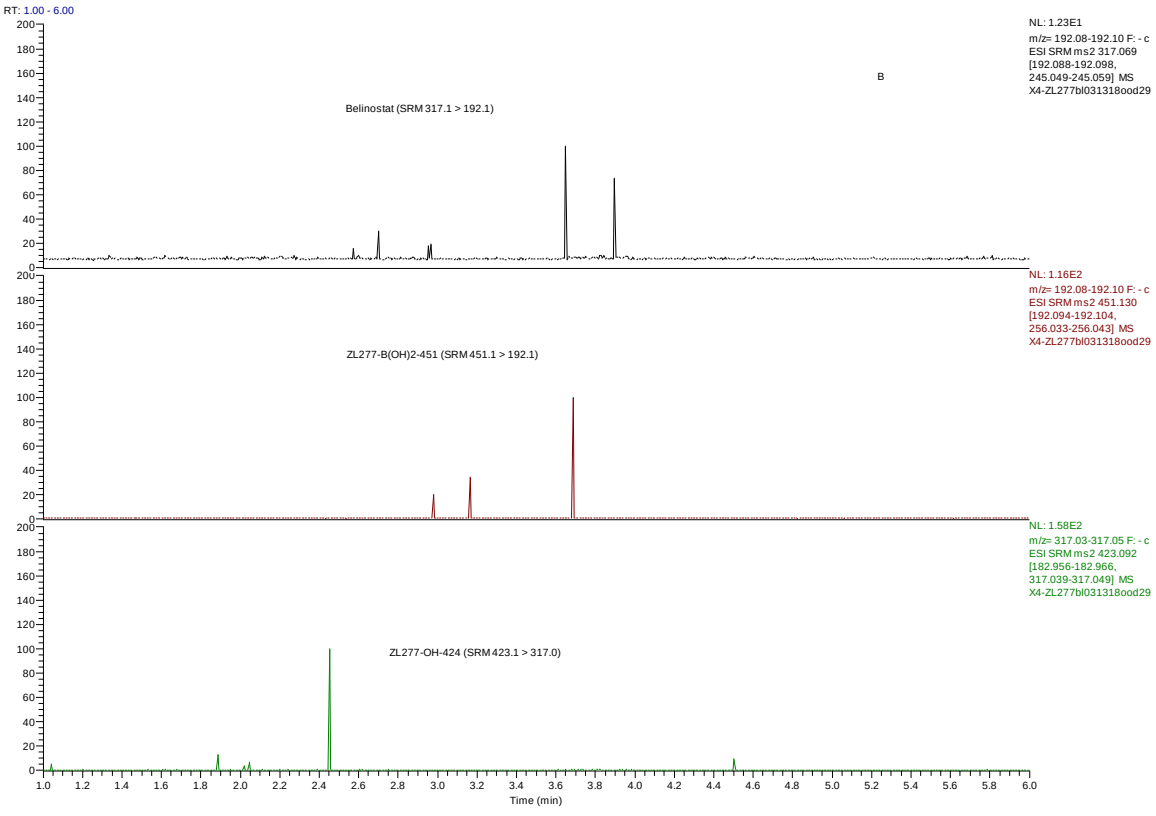
Table S7. Between-run precision and accuracy of Quality control samples for plasma sample measurement during validation.

	Quality Control(pg)	Mean	SD	% CV	% Acc.
Belinostat	795 pg	940	165	17.5	81.7
	6360 pg	6653	639	9.6	95.4
	25440 pg	24486	1433	5.9	96.2
ZL277-B(OH)$_2$-452	1130 pg	1323	195	14.8	82.9
	9040 pg	8516	783	9.2	94.2
	36160 pg	37690	2940	7.8	95.7
ZL277-OH-424	1060 pg	1226	179	14.6	84.4
	8480 pg	8347	792	9.5	98.4
	33920 pg	35524	2757	7.8	95.3

Table S8. Between-run precision and accuracy of Quality control samples for breast sample measurement during validation.

	Quality Control(pg)	Mean	SD	% CV	% Acc.
Belinostat	795 pg	883	110	12.4	88.9
	6360 pg	6902	491	7.1	91.5
	25440 pg	27030	2116	7.8	93.7
ZL277-B(OH)$_2$-452	1130 pg	1346	115	8.5	80.9
	9040 pg	8730	607	7.0	96.6
	36160 pg	36597	582	1.6	98.8
ZL277-OH-424	1060 pg	1155	148	12.8	91.1
	8480 pg	8875	804	9.1	95.3
	33920 pg	34052	957	2.8	99.6


RT: 1.00 - 6.00
A
Belinostat (SRM 317.1 > 192.1)
NL: 1.98E5
m/z= 192.08-192.10 F: - c
ESI SRM ms2 317.069
[192.088-192.098,
245.049-245.059] MS
X4ZL277Tu031418mor59
ZL277-B(OH)2-452 (SRM 451.1 > 192.1)
NL: 5.69E5
m/z= 192.08-192.10 F: - c
ESI SRM ms2 451.130
[192.094-192.104,
256.033-256.043] MS
X4ZL277Tu031418mor59
ZL277-OH-424 (SRM 423.1 > 317.0)
NL: 8.86E3
m/z= 317.03-317.05 F: - c
ESI SRM ms2 423.092
[182.956-182.966,
317.039-317.049] MS
X4ZL277Tu031418mor59
Time (min)


RT: 1.00 - 6.00
B
Belinostat (SRM 317.1 > 192.1)
NL: 1.23E1
m/z= 192.08-192.10 F: - c
ESI SRM ms2 317.069
[192.088-192.098,
245.049-245.059] MS
X4-ZL277bl031318ood29
ZL277-B(OH)2-451 (SRM 451.1 > 192.1)
NL: 1.16E2
m/z= 192.08-192.10 F: - c
ESI SRM ms2 451.130
[192.094-192.104,
256.033-256.043] MS
X4-ZL277bl031318ood29
ZL277-OH-424 (SRM 423.1 > 317.0)
NL: 1.58E2
m/z= 317.03-317.05 F: - c
ESI SRM ms2 423.092
[182.956-182.966,
317.039-317.049] MS
X4-ZL277bl031318ood29
Time (min)

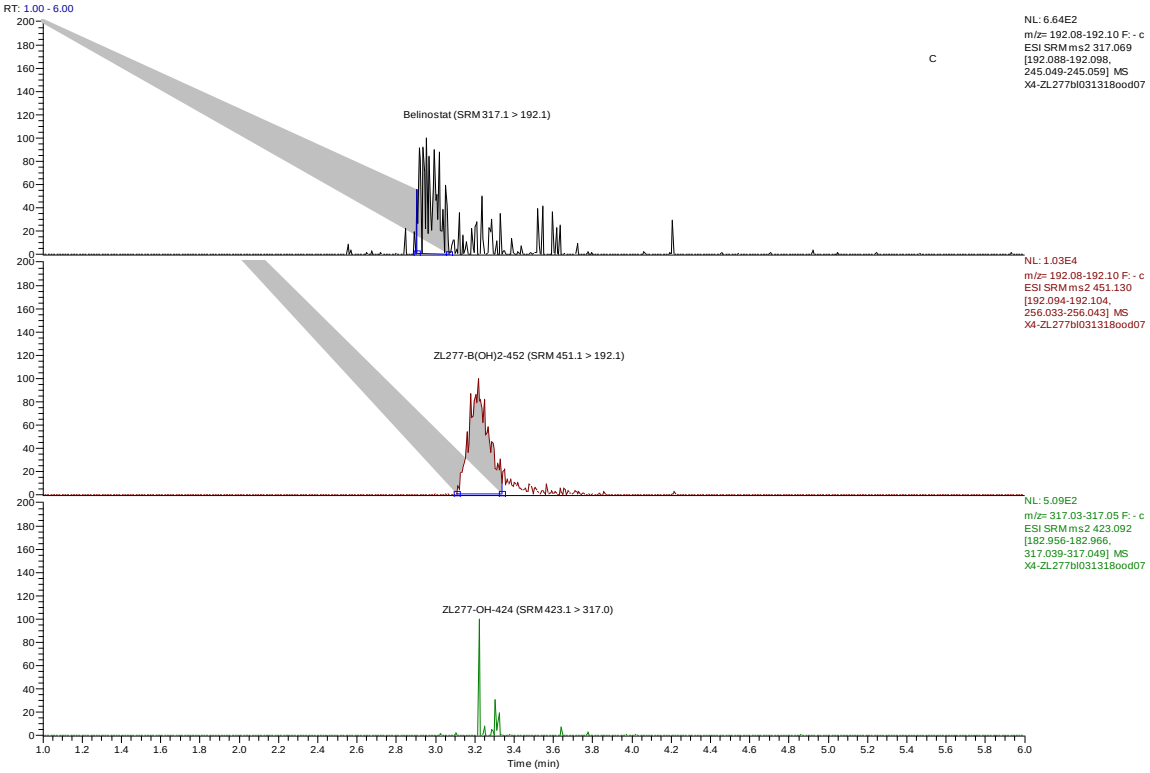


Figure S1. UPLC MS/MS extracted Chromatograms (A) SRM transition of free belinostat, ZL277-B(OH)$_2$-452, and ZL277-OH-424 standard ; (B) a blank mice plasma sample (no belinostat/ZL277); (C) SRM transitions of belinostat, ZL277-B(OH)$_2$-452, and ZL277-OH-424 in mice plasma at 3h after 10mg/kg ZL277 IP injection treatment.

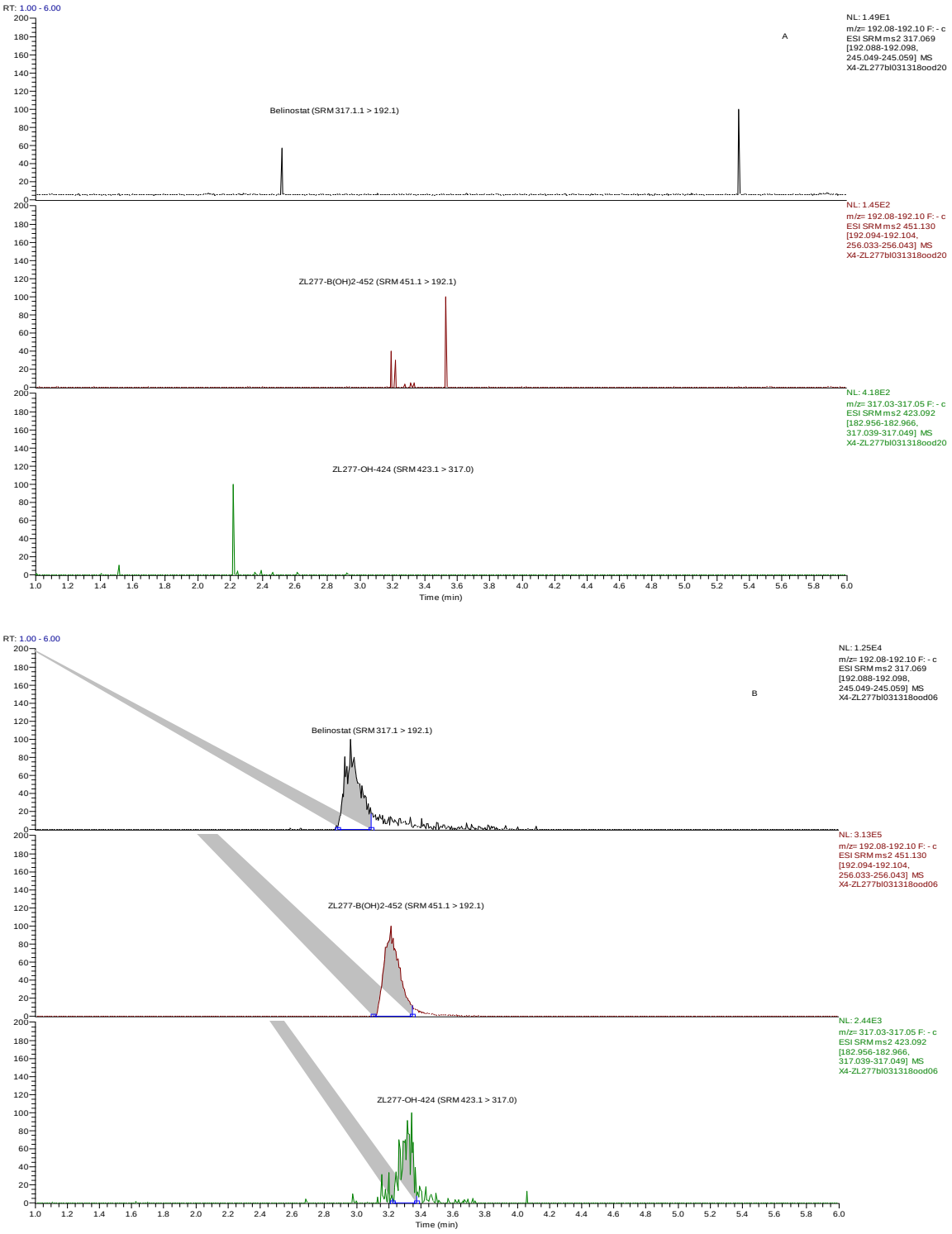


Figure S2. (A) A blank mice breast tumor control sample (without ZL277 or belinostat treatment); (B) SRM transition of belinostat, ZL277-B $(OH)_2$-452, and ZL277-OH-424 in breast tumors at one month after 10 mg/kg ZL277 daily IP injection treatment.

Sample preparation protocols:

(1) Plasma sample preparation: Plasma samples (100 μL) were extracted with methanol. Internal standard (10 μL 0.5 ng/μL trans-tamoxifen-13C2, 15N in methanol), methanol (0.5 mL) were added to each plasma sample. The mixtures were stored at −20 °C overnight. Samples were sonicated for 10 min and then centrifuged on a Heraeus Fresco 21 centrifuge at 14000 rpm. The top liquid layer was dried out and re-suspended in 100 μL of HPLC grade methanol for injection on the TSQ Vantage mass spectrometer instrument.

(2) Feces sample preparation: The collected feces samples were weighed on an OHAUS Explorer balance. The internal standard of trans-tamoxifen-$^{13}C_2$,^{15}N (0.5 ng/μL in methanol) was added to the feces samples based on 10 ng/g feces according to the weight of the feces collected. Methanol was added at the ratio of 4 mL/g feces. Then the samples were homogenized with a Power Gen 125 homogenizer (Fisher Scientific) and stored at -20 °C overnight. After sonicating for 30 min, the samples were centrifuged with a Heraeus Megafuge16R centrifuge at 3000 RPM. The supernatant was dried at room temperature and resuspended with corresponding amount of methanol (200 μL methanol/g feces) for injection on the TSQ Vantage instrument.

(3) Urine sample preparation: Urine samples were thawed at room temperature and vortexed. 200 μL of the sample was transferred to a 1.5 mL Eppendorf microcentrifuge tube followed by adding 200 μL methanol and 10 μL of the 0.5 ng/μL trans-tamoxifen-$^{13}C_2$, ^{15N} in methanol solution. After Vortaxing and centrifuging, the top supernatant was injected on the UPLC-TSQ Vantage mass spectrometer instrument for analysis.