SUPPORTING INFORMATION

ENHANCED PROPERTIES OF A BENZIMIDAZOLE BENZYLPYRAZOLE LYSINE DEMETHYLASE INHIBITOR: MECHANISM-OF-ACTION, BINDING SITE ANALYSIS AND ACTIVITY IN CELLULAR MODELS OF PROSTATE CANCER

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CONTENTS OF SUPPORTING INFORMATION:

Molecular Formula Strings

Compound	Smiles	IC$_{50}$ (µM)
1	OC1=CC(CC(OC)=O)=NN1C2=NC3=CC=CC=C3N2	15
1	OC1=CC(CC(OC)=O)=NN1C2=NC3=CC=CC=C3N2	581
2	OC1=CC(CC(O)=O)=NN1C2=NC3=CC=CC=C3N2	34
3	OC1=C(CCC(OC)=O)C(C)=NN1C2=NC3=CC=CC=C3N2	72
4	OC1=C(C)C(CCC(OC)=O)=NN1C2=NC3=CC=CC=C3N2	106
5	OC1=C(CCC(O)=O)C(C)=NN1C2=NC3=CC=CC=C3N2	274
6	OC1=C(C)C(CCC(O)=O)=NN1C2=NC3=CC=CC=C3N2	437
7	OC1=C(CCC(NC)=O)C(C)=NN1C2=NC3=CC=CC=C3N2	41
8	OC1=C(CCC(N(C)C)=O)C(C)=NN1C2=NC3=CC=CC=C3N2	16
9	OC1=C(CCCNC)C(C)=NN1C2=NC3=CC=CC=C3N2	9
10	OC1=C(CCCN(C)C)C(C)=NN1C2=NC3=CC=CC=C3N2	70
11	OC1=CC(C)=NN1C2=NC3=CC=CC=C3N2	76
12	OC1=C(CC=C)C(C)=NN1C2=NC3=CC=CC=C3N2	24
13	OC1=C(CCC)C(C)=NN1C2=NC3=CC=CC=C3N2	24
14	OC1=C(CC#C)C(C)=NN1C2=NC3=CC=CC=C3N2	5
15	OC1=C(C2=CC=CC=C2)C(C)=NN1C3=NC4=CC=CC=C4N3	0.9
15	OC1=C(C2=CC=CC=C2)C(C)=NN1C3=NC4=CC=CC=C4N3	13
16	OC1=C(CC2=CC=CC=C2)C(C)=NN1C3=NC4=CC=CC=C4N3	27
17	OC1=C(C2=CC=C(C)C=C2)C(C)=NN1C3=NC4=CC=CC=C4N3	27
18	OC1=C(C2=CC=CC=C2)C(CC)=NN1C3=NC4=CC=CC=C4N3	5
19	OC1=C(C2=CC=CC=C2)C(CCCC)=NN1C3=NC4=CC=CC=C4N3	6
20	OC1=C(Br)C=NN1C2=NC3=CC=CC=C3N2	NI*
21	CC1=CC(C)=NN1C2=NC3=CC=CC=C3N2	NI
22	NC1=CC(C)=NN1C2=NC3=CC=CC=C3N2	199
23	NC1=C(C2=CC=CC=C2)C(C)=NN1C3=NC4=CC=CC=C4N3	~160
24	OC1=C(C2=CC=CC=C2)C(C)=NN1C3=NC4=CC=CC=C4S3	35
25	OC1=C(C2=CC=CC=C2)C(C)=NN1C3=NC4=CC=CC=C4N3C	34
26	OC1=C(C2=CC=CC=C2)C(C)=NN1C3=NC=CC=C3	74
27	OC1=C(CC2=CC=CC=C2)C(C)=NN1C3=NC=CC=C3	57
28	OC1=C(CC=C)C(C)=NN1C2=NC=CC=C2	191
29	OC1=C(C2=CC=CC=C2)C(C)=NN1C3=NN=C(Cl)C=C3	68
30	OC1=C(C2=CC=CC=C2)C(C)=NN1C3=NC4=C(C=C(C(O)=O)C=C4)N3	19

*No Inhibition

QC data: Compound 2

Qualitative Compound Report
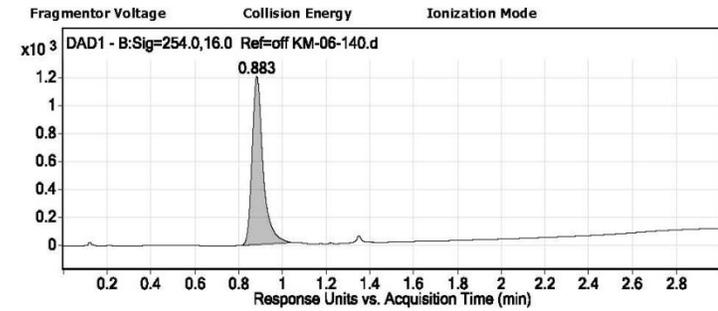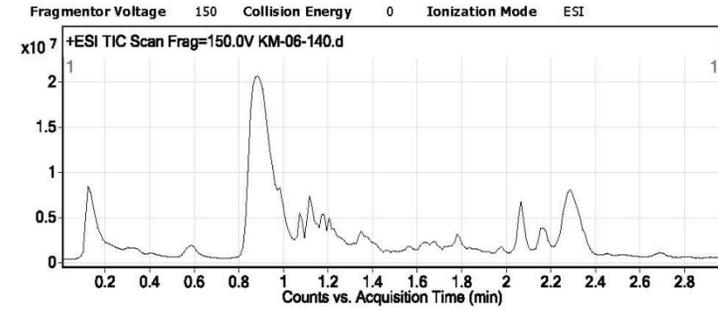
Fragmentor Voltage 150 Collision Energy 0 Ionization Mode ESI

+ESI TIC Scan Frag=150.0V KM-06-140.d

Fragmentor Voltage Collision Energy Ionization Mode

DAD1 - B:Sig=254.0,16.0 Ref=off KM-06-140.d
0.883

User Chromatogram Peak List

Compound Name	Compound Number	RT	Height	Height %	Area	Area %	Area Sum %	Width
Cpd 1: 0.887		1	0.883	1204.16	100	4224.62	100	100	0.247

Compound Table

Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)	Purity Value	Purity Result
Cpd 1: C12 H10 N4 O3	0.887	258.0757	17284	C12 H10 N4 O3	258.0753	1.46	100	Pass

Compound Label	m/z	RT	Algorithm	Mass
Cpd 1: C12 H10 N4 O3	281.0648	0.887	Find By Formula	258.0757

MS Spectrum

Cpd 1: C12 H10 N4 O3: + FBF Spectrum (rt: 0.799-0.832, 0.986-1.075 min) KM-06-140.d Subtract

MS Zoomed Spectrum

Printed at: 1:59 AM on:12/6/2016
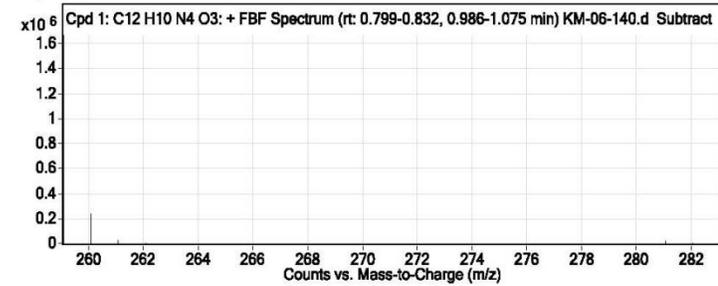
QC Data: Compound 2 continued.

Qualitative Compound Report

Cpd 1: C12 H10 N4 O3: + FBF Spectrum (rt: 0.799-0.832, 0.986-1.075 min) KM-06-140.d Subtract

259.0828 ([C12H10N4O3]+H)+

281.0648 ([C12H10N4O3]+Na)+
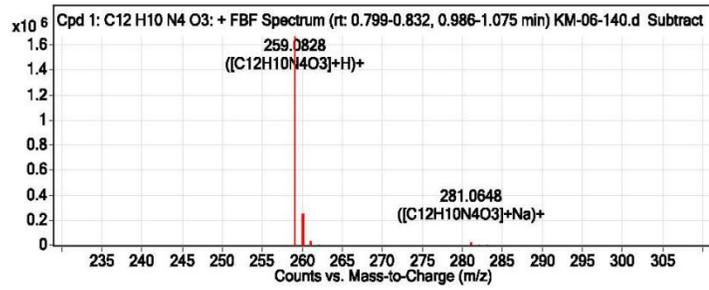
MS Spectrum Peak List

m/z	z	Abund	Formula	Ion
259.0828	1	1669480.75	C12H10N4O3	(M+H)+
260.0865	1	235708.14	C12H10N4O3	(M+H)+
261.0885	1	21914.65	C12H10N4O3	(M+H)+
262.0913	1	1777.77	C12H10N4O3	(M+H)+
281.0648	1	17284.46	C12H10N4O3	(M+Na)+
282.0675	1	2443.94	C12H10N4O3	(M+Na)+
283.072	1	204.28	C12H10N4O3	(M+Na)+

MS Spectrum

Cpd 1: C12 H10 N4 O3: +ESI Scan (rt: 0.799-0.832, 0.986-1.075 min, 13 scans) Frag=150.0V KM-...

259.0828 ([C12H10N4O3]+H)+

MS Zoomed Spectrum

Cpd 1: C12 H10 N4 O3: +ESI Scan (rt: 0.799-0.832, 0.986-1.075 min, 13 scans) Frag=150.0V KM-...

259.0828 ([C12H10N4O3]+H)+

281.0648 ([C12H10N4O3]+Na)+
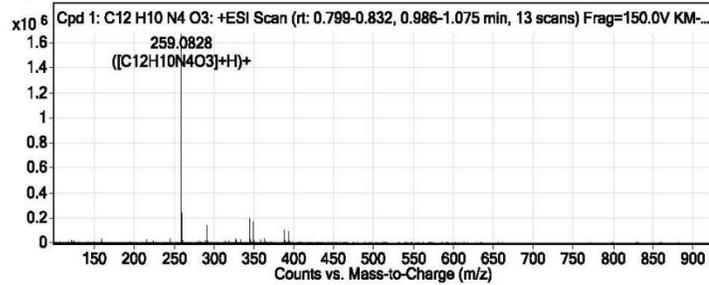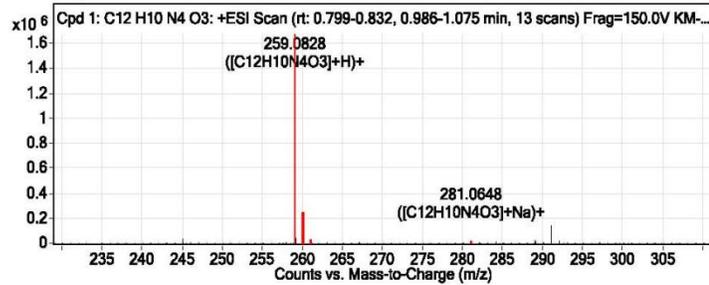
MS Spectrum Peak List

m/z	Calc m/z	Diff(ppm)	z	Abund	Formula	Ion
259.0828	259.0826	-1	1	1669480.75	C12H10N4O3	(M+H)+
260.0865	260.0853	-4.47	1	235708.14	C12H10N4O3	(M+H)+
261.0885	261.0876	-3.69	1	21914.65	C12H10N4O3	(M+H)+
262.0913	262.0899	-5.24	1	1777.77	C12H10N4O3	(M+H)+
281.0648	281.0645	-0.92	1	17284.46	C12H10N4O3	(M+Na)+
282.0675	282.0673	-0.89	1	2443.94	C12H10N4O3	(M+Na)+
283.072	283.0695	-8.89	1	204.28	C12H10N4O3	(M+Na)+

--- End Of Report ---

Printed at: 1:59 AM on:12/6/2016

QC Data: Compound 7

Qualitative Compound Report
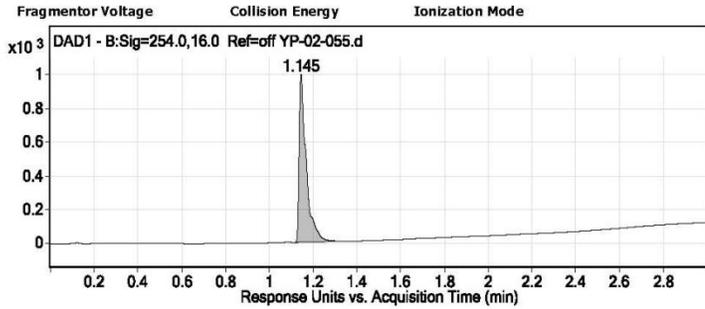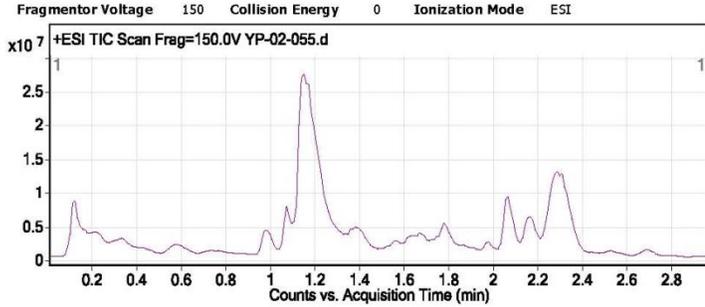
Fragmentor Voltage   150   Collision Energy   0   Ionization Mode   ESI

x10 7   +ESI TIC Scan Frag=150.0V YP-02-055.d

Counts vs. Acquisition Time (min)

Fragmentor Voltage   Collision Energy   Ionization Mode

x10 3   DAD1 - B:Sig=254.0,16.0 Ref=off YP-02-055.d

1.145

Response Units vs. Acquisition Time (min)

User Chromatogram Peak List

Compound Name	Compound Number	RT	Height	Height %	Area	Area %	Area Sum %	Width
Cpd 1: 1.162	1	1.145	994.37	100	2249.11	100	100	0.22

Compound Table

Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)	Purity Value	Purity Result
Cpd 1: C15 H17 N5 O2	1.162	299.1383	1205334	C15 H17 N5 O2	299.1382	0.29	100	Pass

Compound Label	m/z	RT	Algorithm	Mass
Cpd 1: C15 H17 N5 O2	300.1455	1.162	Find By Formula	299.1383

MS Spectrum

x10 6   Cpd 1: C15 H17 N5 O2: + FBF Spectrum (rt: 1.096-1.118, 1.229-1.295 min) YP-02-055.d Subtract

Counts vs. Mass-to-Charge (m/z)
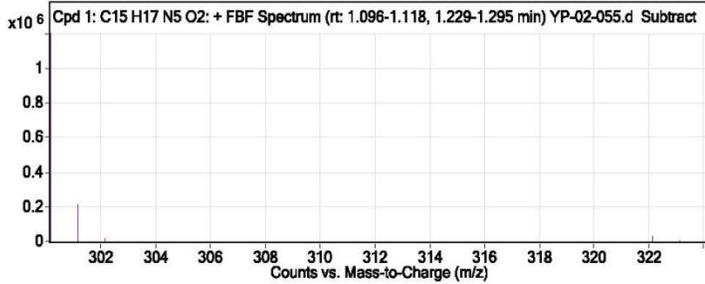
MS Zoomed Spectrum

QC Data: Compound 7 Continued.

Qualitative Compound Report

Cpd 1: C15 H17 N5 O2: + FBF Spectrum (rt: 1.096-1.118, 1.229-1.295 min) YP-02-055.d Subtract

300.1455
([C15H17N5O2]+H)+

322.1278
([C15H17N5O2]+Na)+
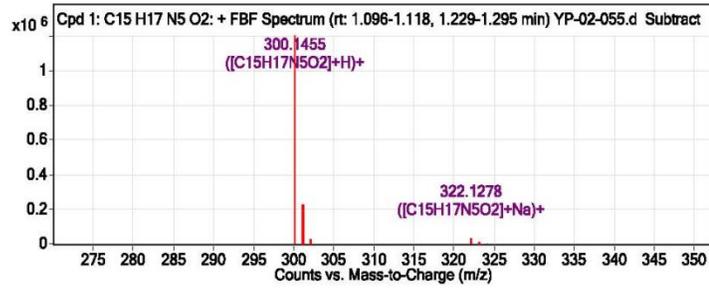
MS Spectrum Peak List

m/z	z	Abund	Formula	Ion
300.1455	1	1205334.38	C15H17N5O2	(M+H)+
301.1487	1	211612.83	C15H17N5O2	(M+H)+
302.1512	1	18919.61	C15H17N5O2	(M+H)+
322.1278	1	32007.41	C15H17N5O2	(M+Na)+
323.1309	1	5530.36	C15H17N5O2	(M+Na)+
324.1371	1	749.17	C15H17N5O2	(M+Na)+

MS Spectrum

Cpd 1: C15 H17 N5 O2: +ESI Scan (rt: 1.096-1.118, 1.229-1.295 min, 10 scans) Frag=150.0V YP-...

300.1455
([C15H17N5O2]+H)+

MS Zoomed Spectrum

Cpd 1: C15 H17 N5 O2: +ESI Scan (rt: 1.096-1.118, 1.229-1.295 min, 10 scans) Frag=150.0V YP-...

300.1455
([C15H17N5O2]+H)+

322.1278
([C15H17N5O2]+Na)+

MS Spectrum Peak List

m/z	Calc m/z	Diff(ppm)	z	Abund	Formula	Ion
300.1455	300.1455	0	1	1205334.38	C15H17N5O2	(M+H)+
301.1487	301.1483	-1.58	1	211612.83	C15H17N5O2	(M+H)+
302.1512	302.1507	-1.42	1	18919.61	C15H17N5O2	(M+H)+
322.1278	322.1274	-1.11	1	32007.41	C15H17N5O2	(M+Na)+
323.1309	323.1302	-2.3	1	5530.36	C15H17N5O2	(M+Na)+
324.1371	324.1327	-13.66	1	749.17	C15H17N5O2	(M+Na)+

--- End Of Report ---

QC Data: Compound 11

Qualitative Compound Report
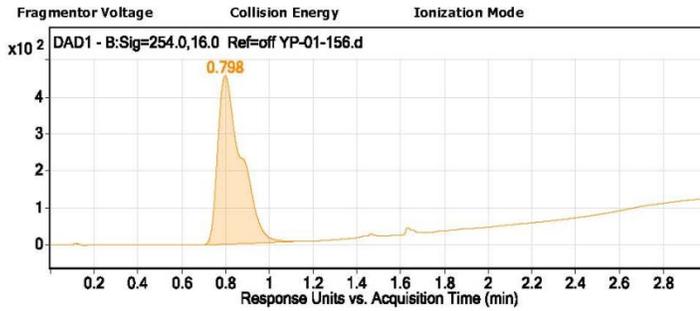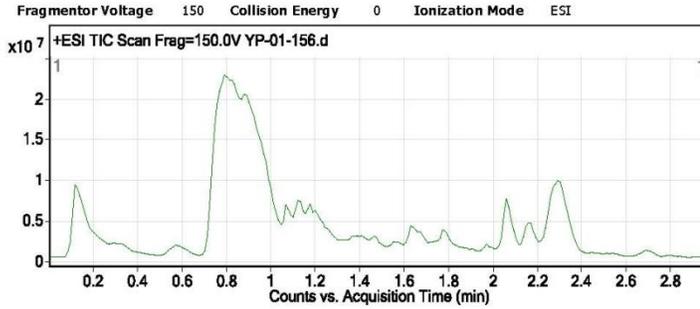
Fragmentor Voltage 150 **Collision Energy** 0 **Ionization Mode** ESI

+ESI TIC Scan Frag=150.0V YP-01-156.d

Fragmentor Voltage **Collision Energy** **Ionization Mode**

DAD1 - B:Sig=254.0,16.0 Ref=off YP-01-156.d

User Chromatogram Peak List

Compound Name	Compound Number	RT	Height	Height %	Area	Area %	Area Sum %	Width
Cpd 1: 0.813	1	0.798	457.28	100	3409.5	100	100	0.443

Compound Table

Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)	Purity Value	Purity Result
Cpd 1: C11 H10 N4 O	0.813	214.0855	36453	C11 H10 N4 O	214.0855	0.21	100	Pass

Compound Label	m/z	RT	Algorithm	Mass
Cpd 1: C11 H10 N4 O	237.075	0.813	Find By Formula	214.0855

MS Spectrum

Cpd 1: C11 H10 N4 O: + FBF Spectrum (rt: 0.692-0.714, 1.023-1.111 min) YP-01-156.d Subtract

MS Zoomed Spectrum

Printed at: 8:03 AM on:11/24/2016
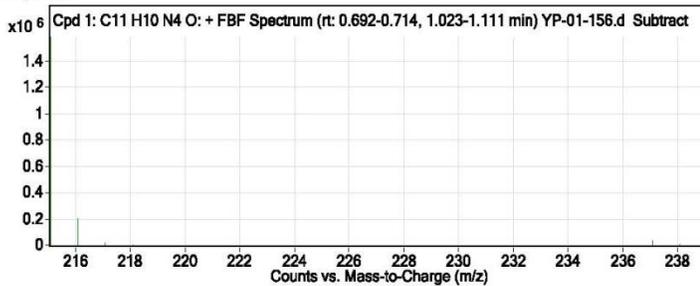
QC Data: Compound 11 continued.

Qualitative Compound Report
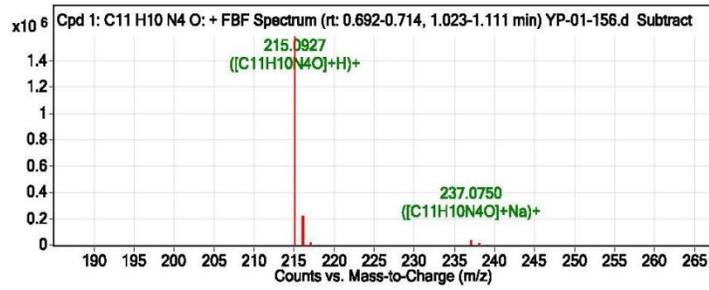
MS Spectrum Peak List

m/z	z	Abund	Formula	Ion
215.0927	1	1583945.63	C11H10N4O	(M+H)+
216.0961	1	203982.45	C11H10N4O	(M+H)+
217.099	1	15169.42	C11H10N4O	(M+H)+
218.0997	1	1053.96	C11H10N4O	(M+H)+
237.075	1	36453.02	C11H10N4O	(M+Na)+
238.0776	1	4451.94	C11H10N4O	(M+Na)+
239.084	1	375.8	C11H10N4O	(M+Na)+

MS Spectrum
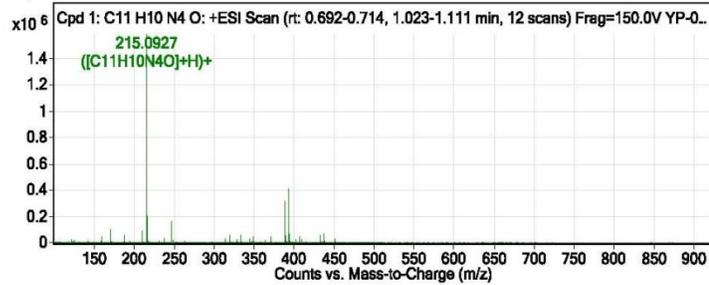
MS Zoomed Spectrum
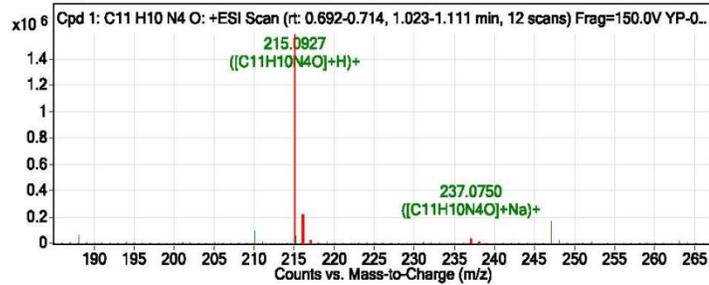
MS Spectrum Peak List

m/z	Calc m/z	Diff(ppm)	z	Abund	Formula	Ion
215.0927	215.0927	0.26	1	1583945.63	C11H10N4O	(M+H)+
216.0961	216.0954	-3.24	1	203982.45	C11H10N4O	(M+H)+
217.099	217.0979	-5.45	1	15169.42	C11H10N4O	(M+H)+
218.0997	218.1002	2.23	1	1053.96	C11H10N4O	(M+H)+
237.075	237.0747	-1.26	1	36453.02	C11H10N4O	(M+Na)+
238.0776	238.0774	-0.96	1	4451.94	C11H10N4O	(M+Na)+
239.084	239.0798	-17.74	1	375.8	C11H10N4O	(M+Na)+

--- End Of Report ---

QC Data: Compound 15

Current Chromatogram(s)

DAD1 A, Sig=300,150 Ref=off (L:\JESSI\YP-03-069_FS.D)

DAD1 B, Sig=254,4 Ref=off (L:\JESSI\YP-03-069_FS.D)

DAD1 C, Sig=220,4 Ref=off (L:\JESSI\YP-03-069_FS.D)

MSD1 TIC, MS File (L:\JESSI\YP-03-069_FS.D)   ES-API, Pos, Scan, Frag: Var

MS Spectrum

*MSD1 SPC, time=1.098 of L:\JES

Max: 848576
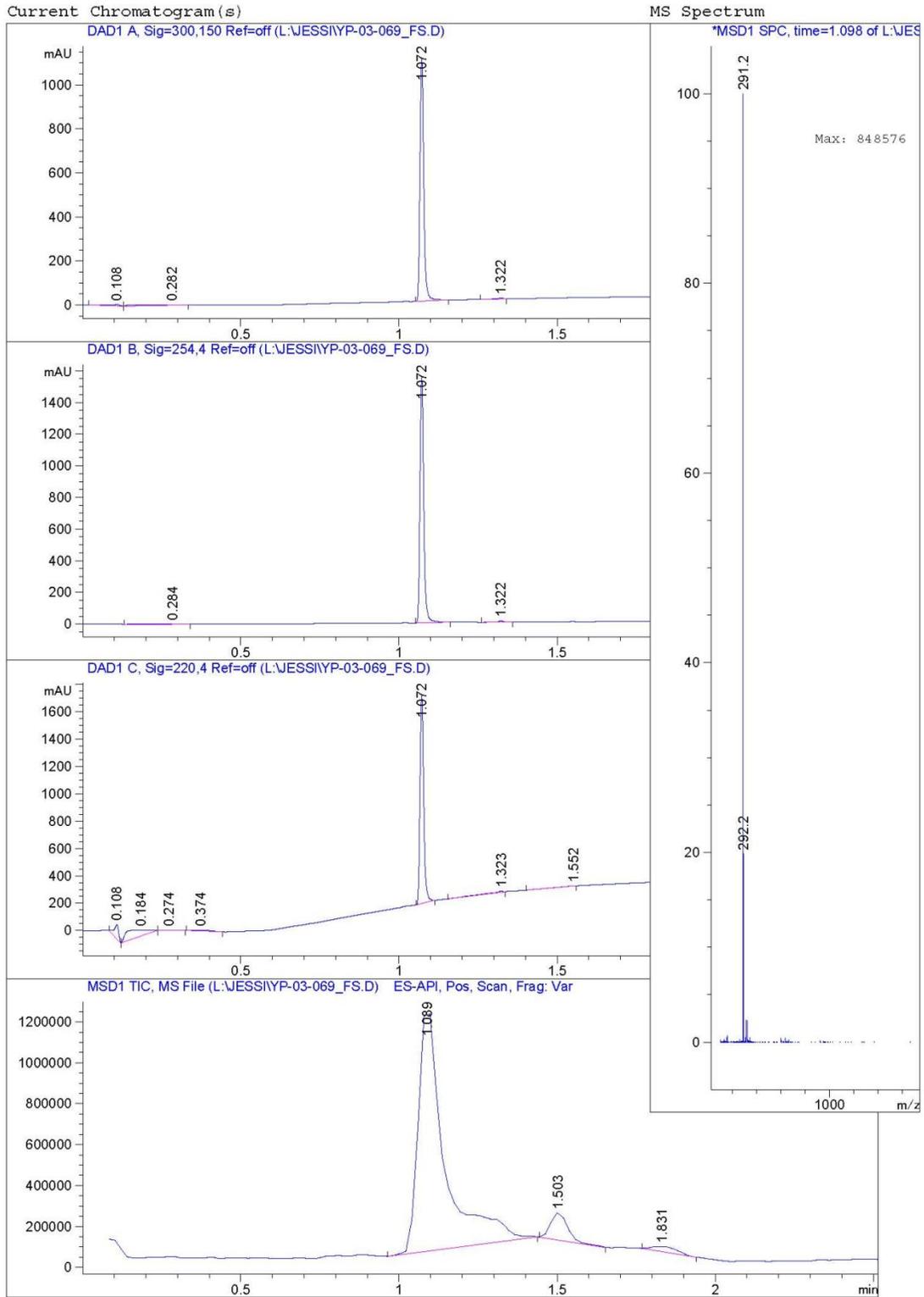
QC Data: Compound 26

Qualitative Compound Report
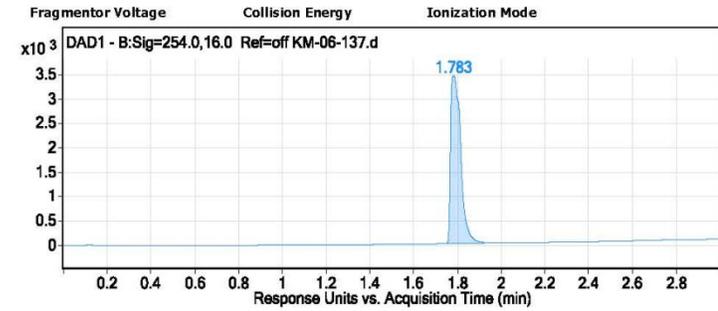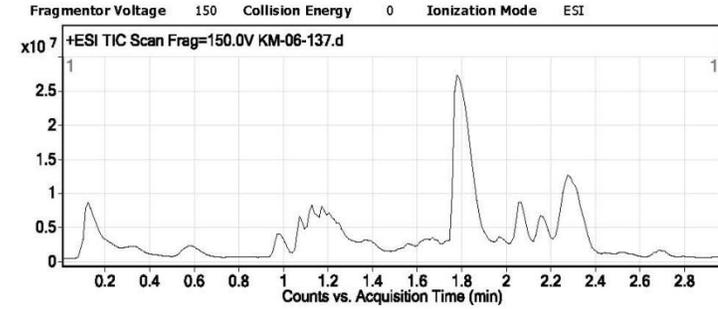
Fragmentor Voltage 150 Collision Energy 0 Ionization Mode ESI

+ESI TIC Scan Frag=150.0V KM-06-137.d

Fragmentor Voltage   Collision Energy   Ionization Mode

DAD1 - B:Sig=254.0,16.0 Ref=off KM-06-137.d

User Chromatogram Peak List

Compound Name	Compound Number		RT	Height	Height %	Area	Area %	Area Sum %	Width
Cpd 1: 1.801		1	1.783	3446.2	100	11072.13	100	100	0.278

Compound Table

Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)	Purity Value	Purity Result
Cpd 1: C15 H13 N3 O	1.801	251.1065	1005455	C15 H13 N3 O	251.1059	2.66	100	Pass

Compound Label	m/z	RT	Algorithm	Mass
Cpd 1: C15 H13 N3 O	252.1137	1.801	Find By Formula	251.1065

MS Spectrum

Cpd 1: C15 H13 N3 O: + FBF Spectrum (rt: 1.724-1.746, 1.878-1.944 min) KM-06-137.d Subtract

MS Zoomed Spectrum

S10
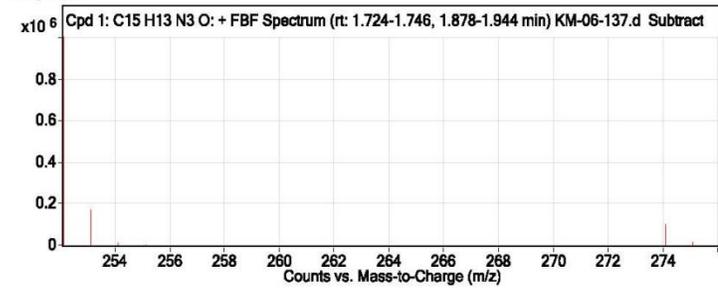
QC Data: Compound 26 continued.

Qualitative Compound Report

Cpd 1: C15 H13 N3 O: + FBF Spectrum (rt: 1.724-1.746, 1.878-1.944 min) KM-06-137.d Subtract

252.1137 ([C15H13N3O]+H)+
274.0961 ([C15H13N3O]+Na)+
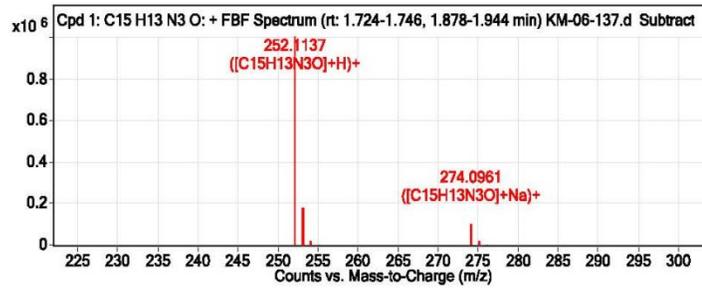
MS Spectrum Peak List

m/z	z	Abund	Formula	Ion
252.1137	1	1005454.63	C15H13N3O	(M+H)+
253.1173	1	169138.77	C15H13N3O	(M+H)+
254.1199	1	12746.42	C15H13N3O	(M+H)+
255.1221	1	934.2	C15H13N3O	(M+H)+
274.0961	1	100997.13	C15H13N3O	(M+Na)+
275.0989	1	14722.76	C15H13N3O	(M+Na)+
276.1013	1	1340.76	C15H13N3O	(M+Na)+

MS Spectrum

Cpd 1: C15 H13 N3 O: +ESI Scan (rt: 1.724-1.746, 1.878-1.944 min, 10 scans) Frag=150.0V KM-0...

252.1137 ([C15H13N3O]+H)+

MS Zoomed Spectrum

Cpd 1: C15 H13 N3 O: +ESI Scan (rt: 1.724-1.746, 1.878-1.944 min, 10 scans) Frag=150.0V KM-0...

252.1137 ([C15H13N3O]+H)+
274.0961 ([C15H13N3O]+Na)+
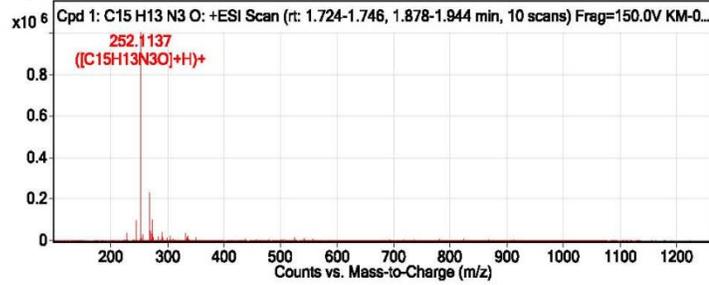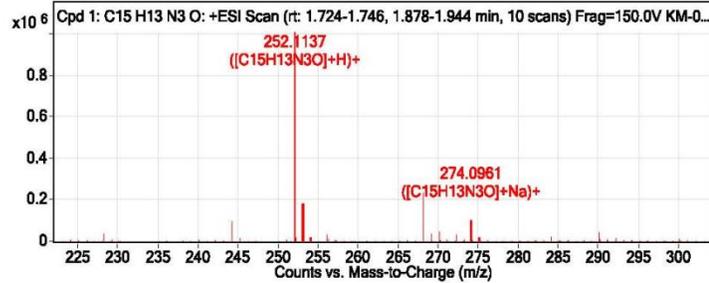
MS Spectrum Peak List

m/z	Calc m/z	Diff(ppm)	z	Abund	Formula	Ion
252.1137	252.1131	-2.11	1	1005454.63	C15H13N3O	(M+H)+
253.1173	253.1161	-4.82	1	169138.77	C15H13N3O	(M+H)+
254.1199	254.1189	-3.99	1	12746.42	C15H13N3O	(M+H)+
255.1221	255.1215	-2.26	1	934.2	C15H13N3O	(M+H)+
274.0961	274.0951	-3.85	1	100997.13	C15H13N3O	(M+Na)+
275.0989	275.0981	-2.87	1	14722.76	C15H13N3O	(M+Na)+
276.1013	276.1008	-1.59	1	1340.76	C15H13N3O	(M+Na)+

--- End Of Report ---

Printed at: 8:03 AM on:11/24/2016

QC Data: Compound 29

Qualitative Analysis Report

User Chromatograms

Fragmentor Voltage 175   Collision Energy 0   Ionization Mode ESI

+ESI TIC Scan Frag=175.0V YP-03-097.d
Counts (%) vs. Acquisition Time (min)

DAD1 - B:Sig=254,16 YP-03-097.d
1.269
0.144
Response Units (%) vs. Acquisition Time (min)
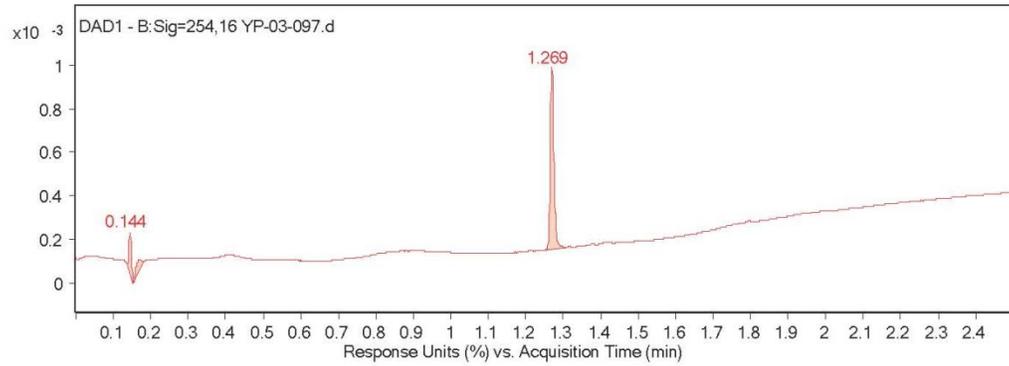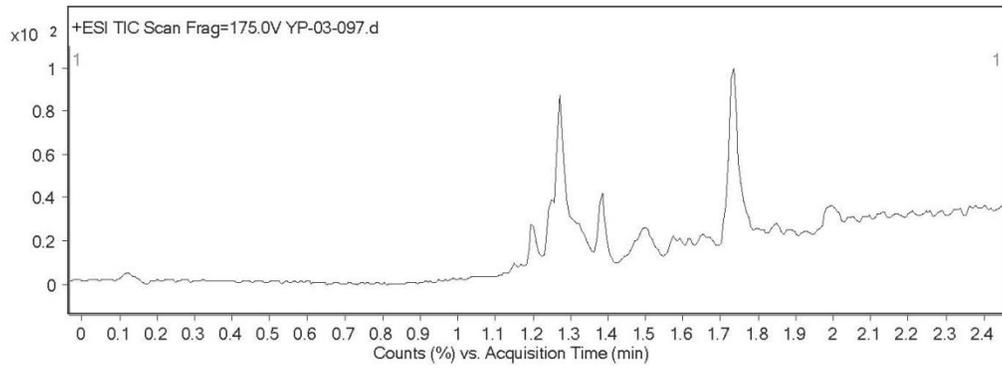
User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
175	0	ESI

QC Data: Compound 29 continued.

Qualitative Analysis Report

+ESI Scan (rt: 1.277 min) Frag=175.0V YP-03-097.d

x10 5

287.0692

886.4130

Counts vs. Mass-to-Charge (m/z)
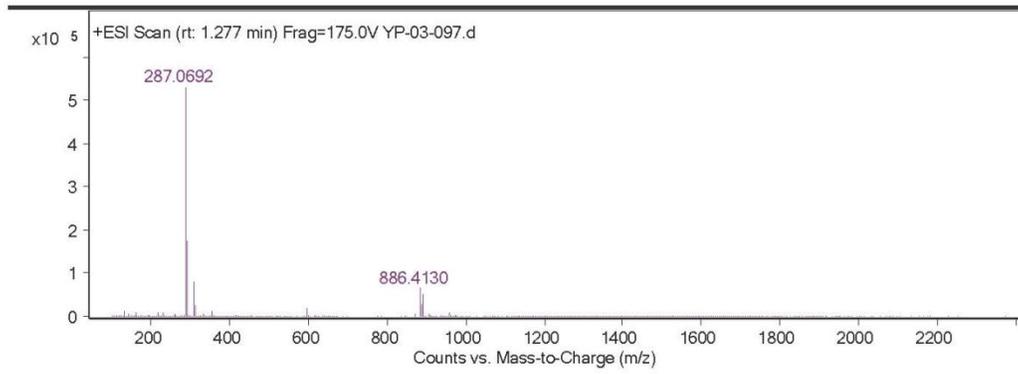
--- End Of Report ---

QC Data: Compound 30

Current Chromatogram(s)

DAD1 A, Sig=300,150 Ref=off (K:\2016\JESSI\YP-02\141-160\145\YP-02-145_RT_UEN.D)

DAD1 B, Sig=254,4 Ref=off (K:\2016\JESSI\YP-02\141-160\145\YP-02-145_RT_UEN.D)

DAD1 C, Sig=220,4 Ref=off (K:\2016\JESSI\YP-02\141-160\145\YP-02-145_RT_UEN.D)

MSD1 TIC, MS File (K:\2016\JESSI\YP-02\141-160\145\YP-02-145_RT_UEN.D)   ES-API, Pos, Scan, Frag: Var

MS Spectrum

*MSD1 SPC, time=1.007:1.065 of K:\2016\JESSI\YP-02\141-160\145\YP-02-145_RT_UEN.D   ES-API, Pos, Scan, Frag: Var

Max: 151286

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S14

Table S1. Synthetic Derivatives of a Benzimidazole Pyrazole-based JmjC-KDM Inhibitor

Benzimidazole pyrazoles	Pyridine pyrazoles	Pyridazine-substituted Variant	Carboxy-substituted Variant
cmpds **1-25**	cmpds **26-28**	cmpd **29**	cmpd **30**

	Benzimidazole pyrazole-based scaffold				Best Fit Parameters					
cmpd	X	R1	R2	R3	IC$_{50}$ μM[a]	IC$_{50}$ 95% CI μM[b]	top[c]	bottom[d]	Hill slope[e]	residual[f]
1[g]	NH	CH$_2$CO$_2$Me	H	OH	15	12–19	105%	2%	-1.1	2%
1[h]	NH	CH$_2$CO$_2$Me	H	OH	581[i]	486–696	100%	0%	0.8	10%
2	NH	CH$_2$CO$_2$H	H	OH	347	293–411	96%	-5%	-1.6	1%
3	NH	Me	CH$_2$CH$_2$CO$_2$Me	OH	72	68–77	96%	0%	-2.3	1%
4	NH	CH$_2$CH$_2$CO$_2$Me	Me	OH	106	97–117	101%	2%	-2.1	1%
5	NH	Me	CH$_2$CH$_2$CO$_2$H	OH	274	182–414	97%	0%	-0.9	7%
6	NH	CH$_2$CH$_2$CO$_2$H	Me	OH	437	328–581	95%	0%	-1.6	1%
7	NH	Me	CH$_2$CH$_2$CONHMe	OH	41	37–46	104%	1%	-1.4	1%
8	NH	Me	CH$_2$CH$_2$CONMe$_2$	OH	16	13–18	96%	1%	-1.6	1%
9	NH	Me	CH$_2$CH$_2$CH$_2$NHMe	OH	9	6–15	97%	-3%	-0.6	5%
10	NH	Me	CH$_2$CH$_2$CH$_2$NMe$_2$	OH	70	59–84	94%	-5%	-1.8	1%
11	NH	Me	H	OH	76	62–93	97%	-1%	-1.2	0%
12	NH	Me	allyl	OH	24	22–27	93%	3%	-1.9	4%
13	NH	Me	n-propyl	OH	24	22–27	99%	5%	-1.6	4%
14	NH	Me	propargyl	OH	5	4–6	100%	2%	-1.1	2%
15[g]	NH	Me	Ph	OH	0.9	0.6–1.1	105%	3%	-0.9	1%
15[h]	NH	Me	Ph	OH	13	12–14	92%	4%	4.2	2%
16	NH	Me	Bn	OH	27	21–35	104%	3%	-0.9	3%
17	NH	Me	*para*-tol	OH	27	24–31	95%	0%	-2.0	4%
18	NH	Et	Ph	OH	5	4–6	100%	1%	-1.6	1%
19	NH	Bu	Ph	OH	6	5.6–6.0	100%	2%	-2.2	0.3%
20	NH	H	Br	H	NI[j]	NA[k]	NA	NA	NA	~100%
21	NH	Me	H	Me	NI	NA	NA	NA	NA	~100%
22	NH	Me	H	NH$_2$	199	160–248	104%	5%	-1.4	2%
23	NH	Me	Ph	NH$_2$	~160	107–239	100%	0%	-0.7	0%
24	S	Me	Ph	OH	35	30–41	95%	5%	-1.5	5%
25	NMe	Me	Ph	OH	34	26–46	96%	5%	-2.7	3%

	Pyridine pyrazole-based scaffold				Best Fit Parameters			
cmpd	R1	R2	IC$_{50}$ μM	IC$_{50}$ 95% CI μM	top	bottom	Hill slope	residual
26	Me	Ph	74	62–89	95%	1%	-2.6	0%
27	Me	Bn	57	49–67	96%	-1.6%	-1.4	1%
28	Me	allyl	191	81–450	100%	2%	-0.8	2%

	Pyridazine-substituted Variant		Best Fit Parameters			
cmpd	see structure above	IC$_{50}$ μM	IC$_{50}$ 95% CI μM	top	bottom	Hill slope	residual
29		68	61–76	100%	3%	-1.5	1%

|	Carboxy-substituted Variant		Best Fit Parameters			
cmpd	see structure above	IC$_{50}$ μM	IC$_{50}$ 95% CI μM	top	bottom	Hill slope	residual
30		19	15–24	107%	-2%	-0.9	0%

[a]Values calculated from kinetic data using the log(inhibitor) vs. response -- Variable slope inhibition model in GraphPad Prism. [b]Fitting error reported as a 95% confidence interval. [c]Highest level of enzyme activity fit as a variable in the inhibition model. [d]Lowest level of enzyme activity fit as a variable in the inhibition model. [e]Steepness, or hill slope of the dose response curve. [f]Level of enzyme activity remaining at the highest inhibitor concentration tested. [g]Values from the FDH-based assay. [h]Values from the CTH ELISA-based assay. [i]Data reproduced from Carter *et al.* (2017): values of IC$_{50}$ = 12.5 μM and 800 μM when fit to a biphasic inhibition model. [j]No inhibition seen at the highest concentration of inhibitor tested. [k]Not applicable given a lack of inhibitor efficacy.

Table S2. Crystallographic Data

	KDM4A with cmpd **26** PDB code 6G5W	KDM4A with cmpd **30** PDB code 6G5X
resolution range (Å)	46.57 - 1.826 (1.892 - 1.826)*	46.84 - 1.78 (1.843 - 1.78)
space group	P 2_1 2_1 2	P 2_1 2_1 2
unit cell a, b, c (Å), α, β, γ (°)	99.36 148.57 56.39 90 90 90	99.14 148.61 56.91 90 90 90
total np. of reflections	432868	478462
No. of unique reflections	74525 (7156)	81224 (7904)
multiplicity	5.8	5.9
completeness (%)	99.50 (97.00)	99.75 (98.15)
mean I/sigma(I)	15.78 (1.70)	21.54 (1.69)
Wilson B-factor	30.75	28.64
R-meas (%)	6.8 (100.2)	5.6 (105.3)
$CC_{1/2}$	99.9 (71.1)	100 (69.9)
R-work (%)	17.5 (27.9)	16.8 (27.8)
R-free (%)	21 (032.2)	20.6 (31.7)
number of non-hydrogen atoms	6250	6389
macromolecules	5723	5756
ligands	77	86
water	443	538
protein residues	692	696
RMS(bonds) (Å)	0.012	0.011
RMS(angles) (°)	1.29	1.36
Ramachandran favored (%)	98	98
Ramachandran allowed (%)	2	2
Ramachandran outliers (%)	0	0
clashscore	1.66	1.22
average B-factor (Å^2)	44.60	39.30
macromolecules	44.60	39.00
ligands	46.80	36.80
solvent	44.70	42.80

*Values in parentheses are for the respective highest resolution shell.
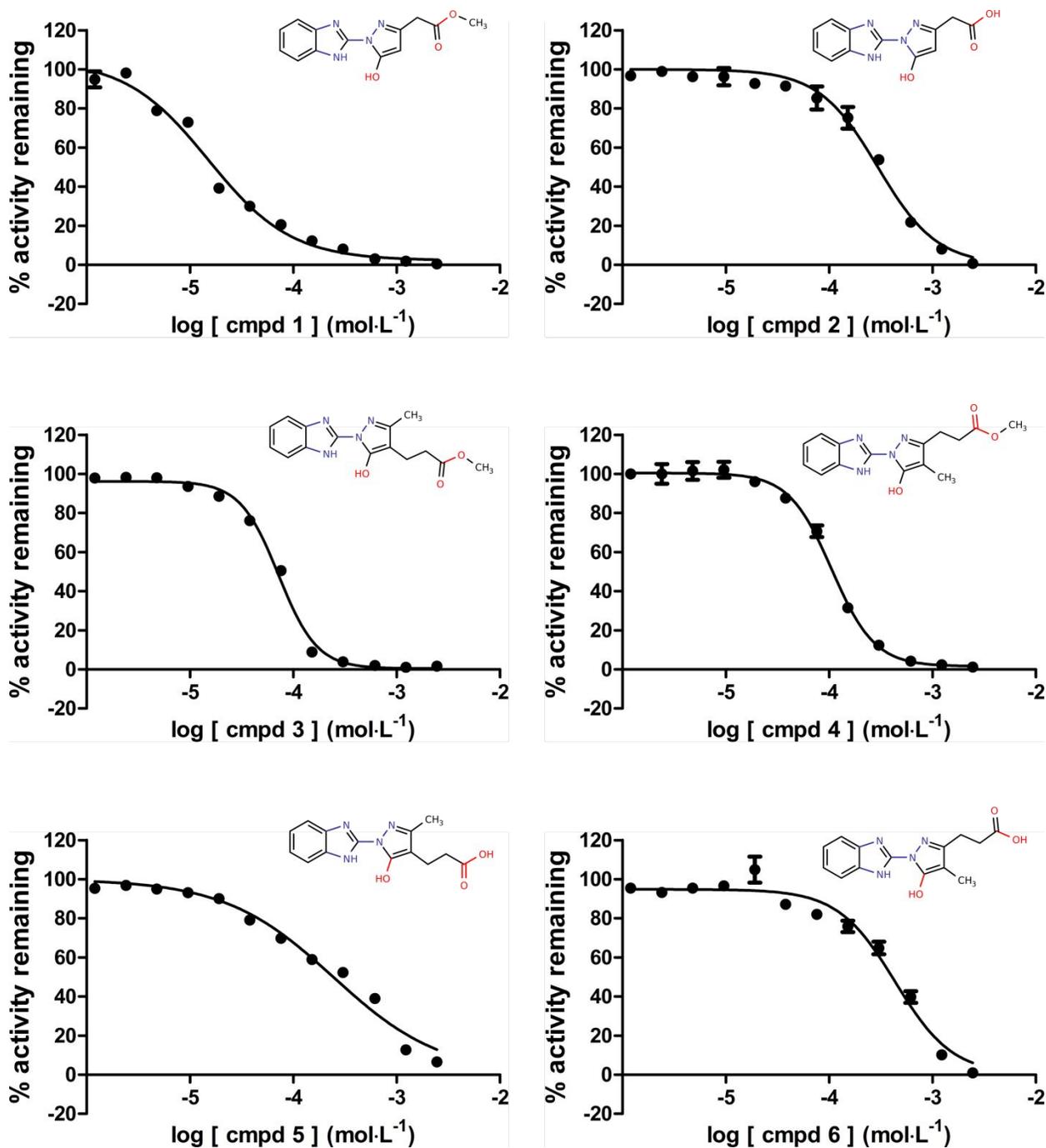
Figure S1. Inhibitory properties of selected KDM4 inhibitors, designed to assess H-bonding capacity within the KDM4E active site. Inhibition profiles resulting from FDH-based, KDM4E activity assays are depicted with best fit lines indicated using the log(inhibitor) vs. response -- Variable slope inhibition model in GraphPad Prism. The corresponding compound structures are depicted above each plot. Inhibitory profiles were assessed relative to data measured from the original HTS hit, compound **1** (top left panel).
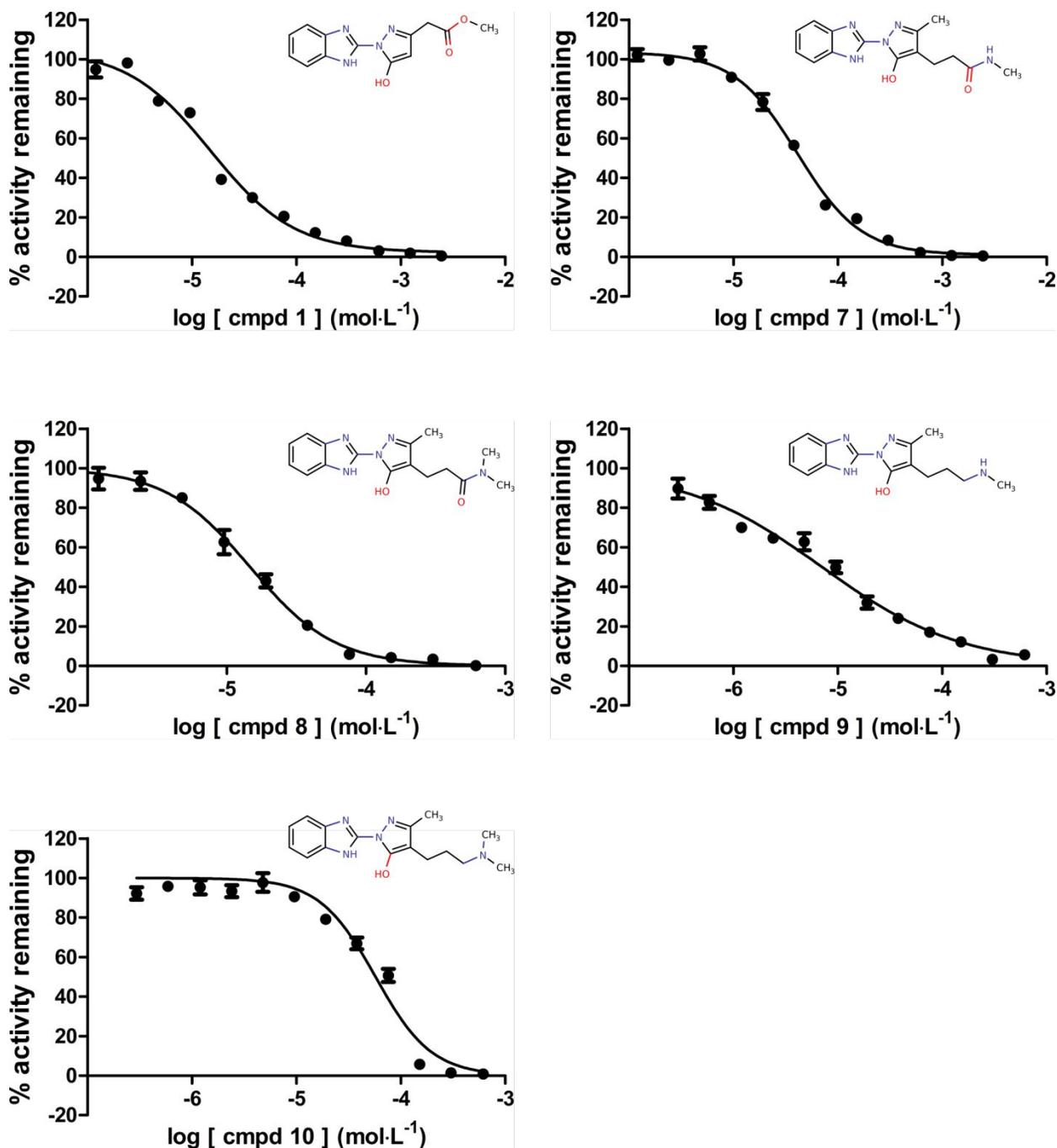
Figure S2. Inhibitory properties of selected KDM4 inhibitors, designed to assess substituted amine binding within the KDM4E active site. Inhibition profiles resulting from FDH-based, KDM4E activity assays are depicted with best fit lines indicated using the log(inhibitor) vs. response -- Variable slope inhibition model in GraphPad Prism. The corresponding compound structures are depicted above each plot. Inhibitory profiles were assessed relative to data measured from the original HTS hit, compound **1** (top left panel).
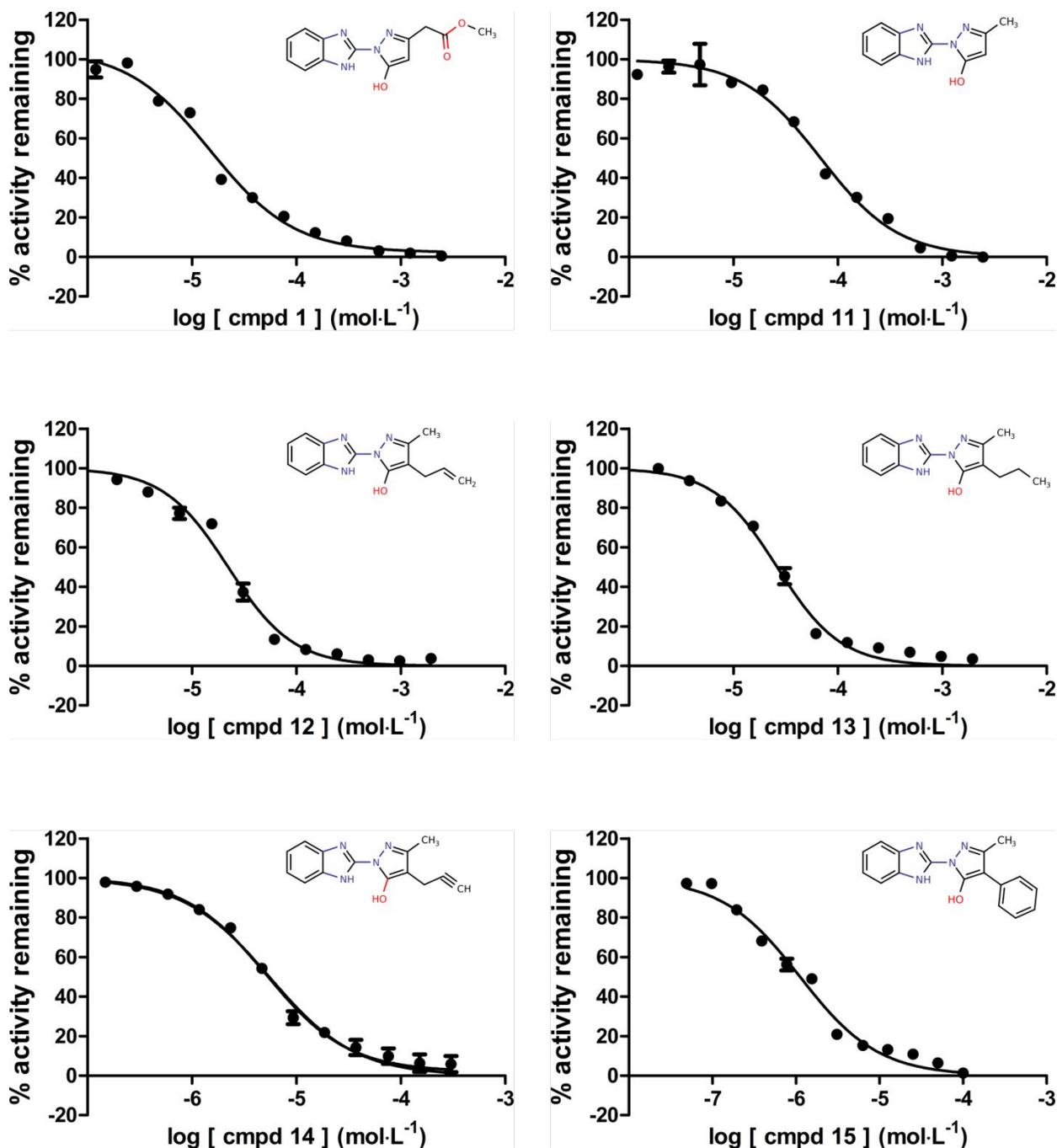
Figure S3. Inhibitory properties of selected KDM4 inhibitors, designed to assess inhibitor sidechain polarity at the R2 scaffold position. Inhibition profiles resulting from FDH-based, KDM4E activity assays are depicted with best fit lines indicated using the log(inhibitor) vs. response -- Variable slope inhibition model in GraphPad Prism. The corresponding compound structures are depicted above each plot. Inhibitory profiles were assessed relative to data measured from the original HTS hit, compound **1** (top left panel).
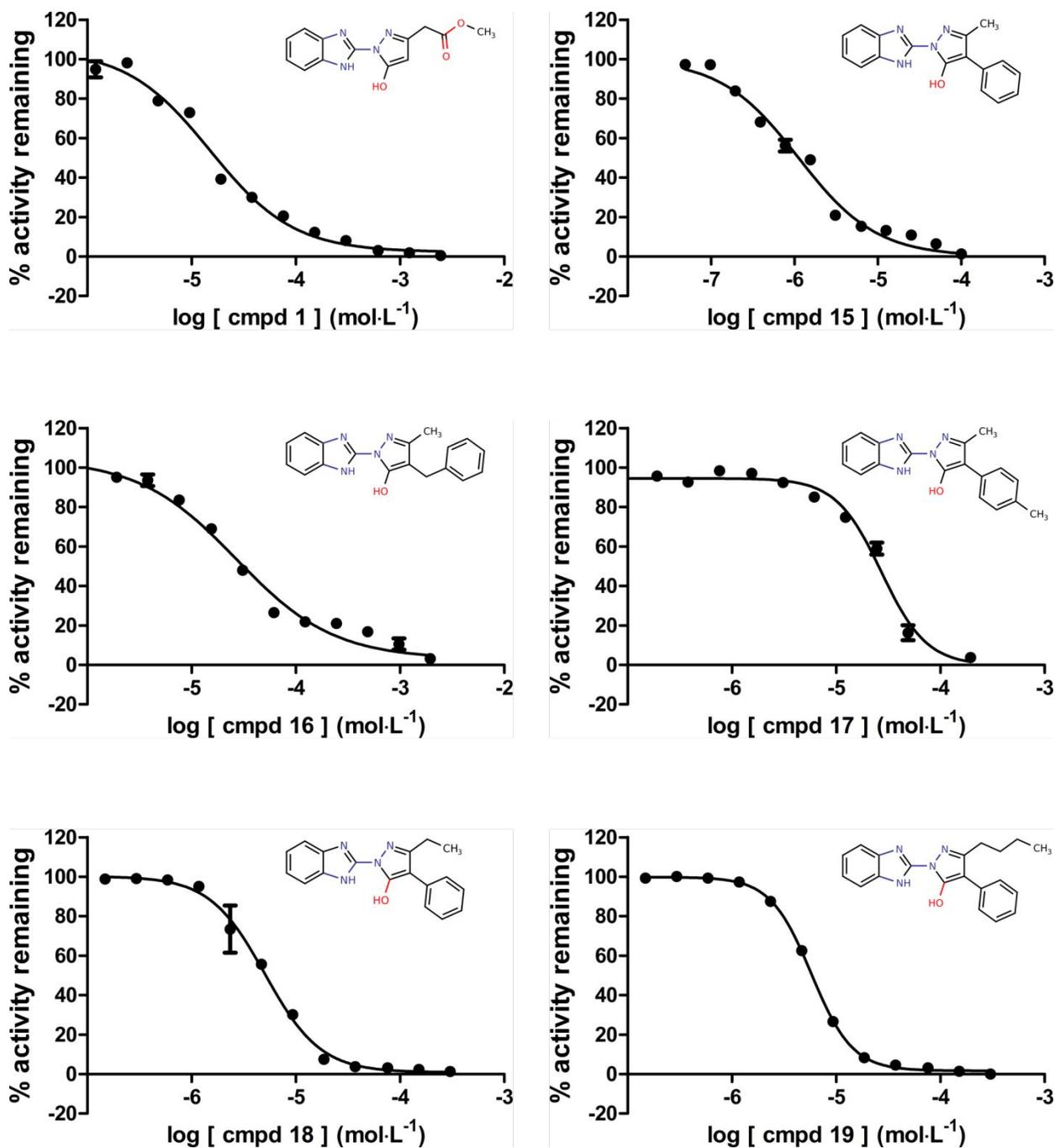
Figure S4. Inhibitory properties of selected KDM4 inhibitors, designed to further assess inhibitor sidechain polarity at R1 and R2 scaffold positions. Inhibition profiles resulting from FDH-based, KDM4E activity assays are depicted with best fit lines indicated using the log(inhibitor) vs. response -- Variable slope inhibition model in GraphPad Prism. The corresponding compound structures are depicted above each plot. Inhibitory profiles were assessed relative to data measured from the original HTS hit (compound **1**, top left) and the most potent inhibitor tested (compound **15**, top right).
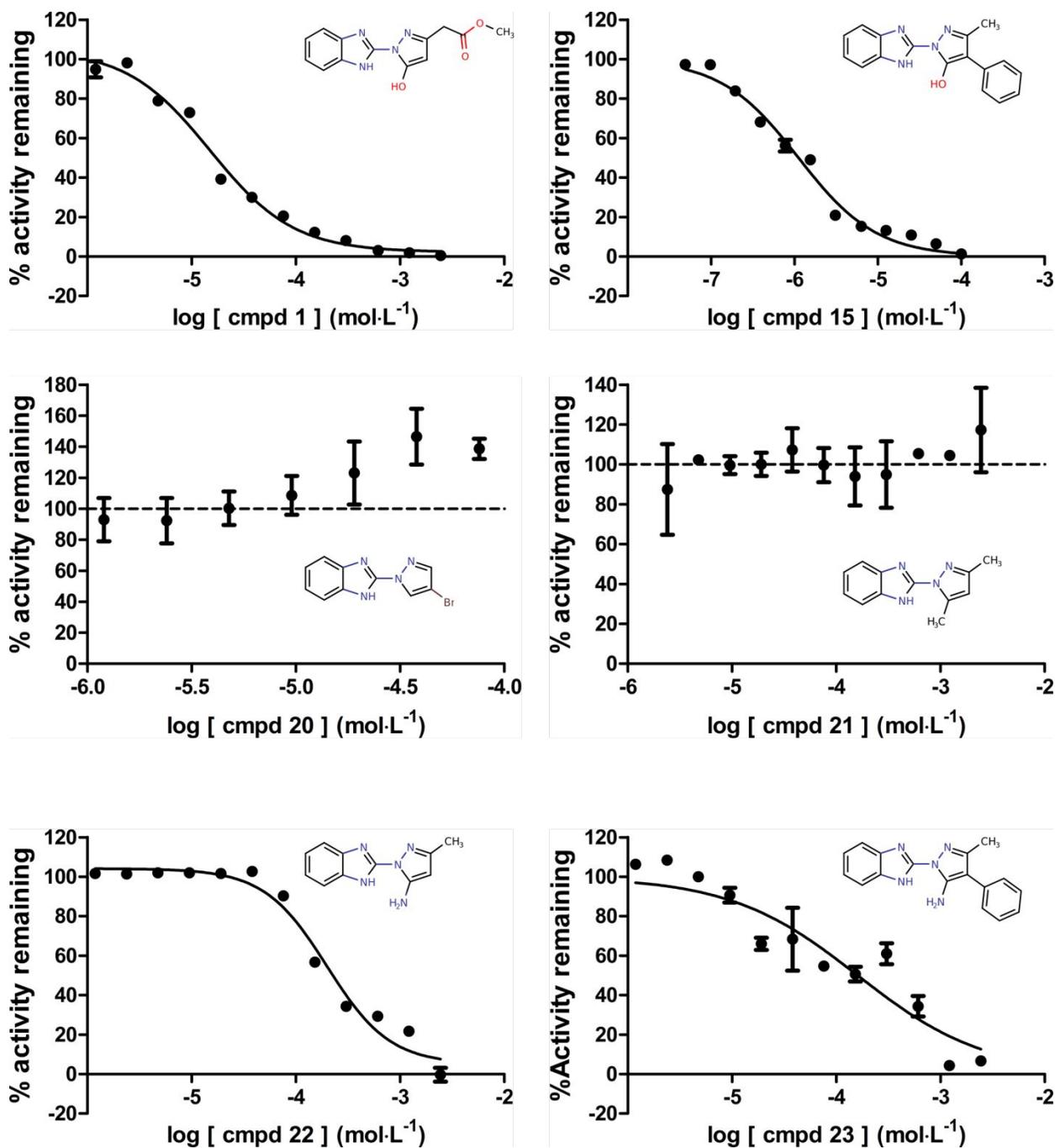
Figure S5. Inhibitory properties of focused KDM4 inhibitors, designed or selected to characterize substitutions of the pyrazole hydroxyl moiety. Inhibition profiles resulting from FDH-based, KDM4E activity assays are depicted with best fit lines indicated using the log(inhibitor) vs. response -- Variable slope inhibition model in GraphPad Prism. The corresponding compound structures are depicted within each panel. Inhibitory profiles were assessed relative to data measured from the original HTS hit (compound **1**, top left) and the most potent inhibitor tested (compound **15**, top right).
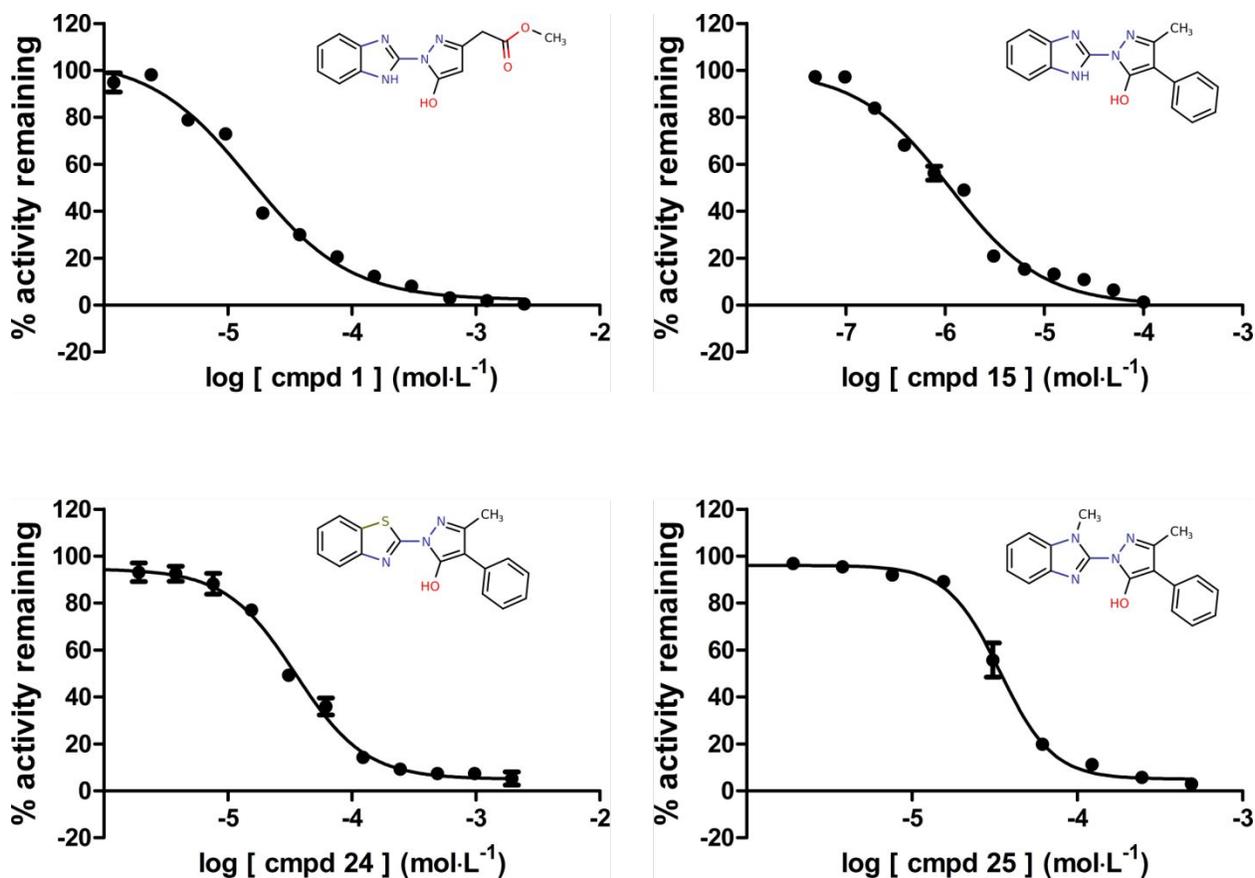
Figure S6. Inhibitory properties of selected KDM4 inhibitors, designed to assess the potential of active site Fe^{2+} chelation via the scaffold's bidentate chelation motif. Inhibition profiles resulting from FDH-based, KDM4E activity assays are depicted with best fit lines indicated using the log(inhibitor) vs. response -- Variable slope inhibition model in GraphPad Prism. The corresponding compound structures are depicted within each panel. Inhibitory profiles were assessed relative to data measured from the original HTS hit (compound **1**, top left) and the most potent inhibitor tested (compound **15**, top right).
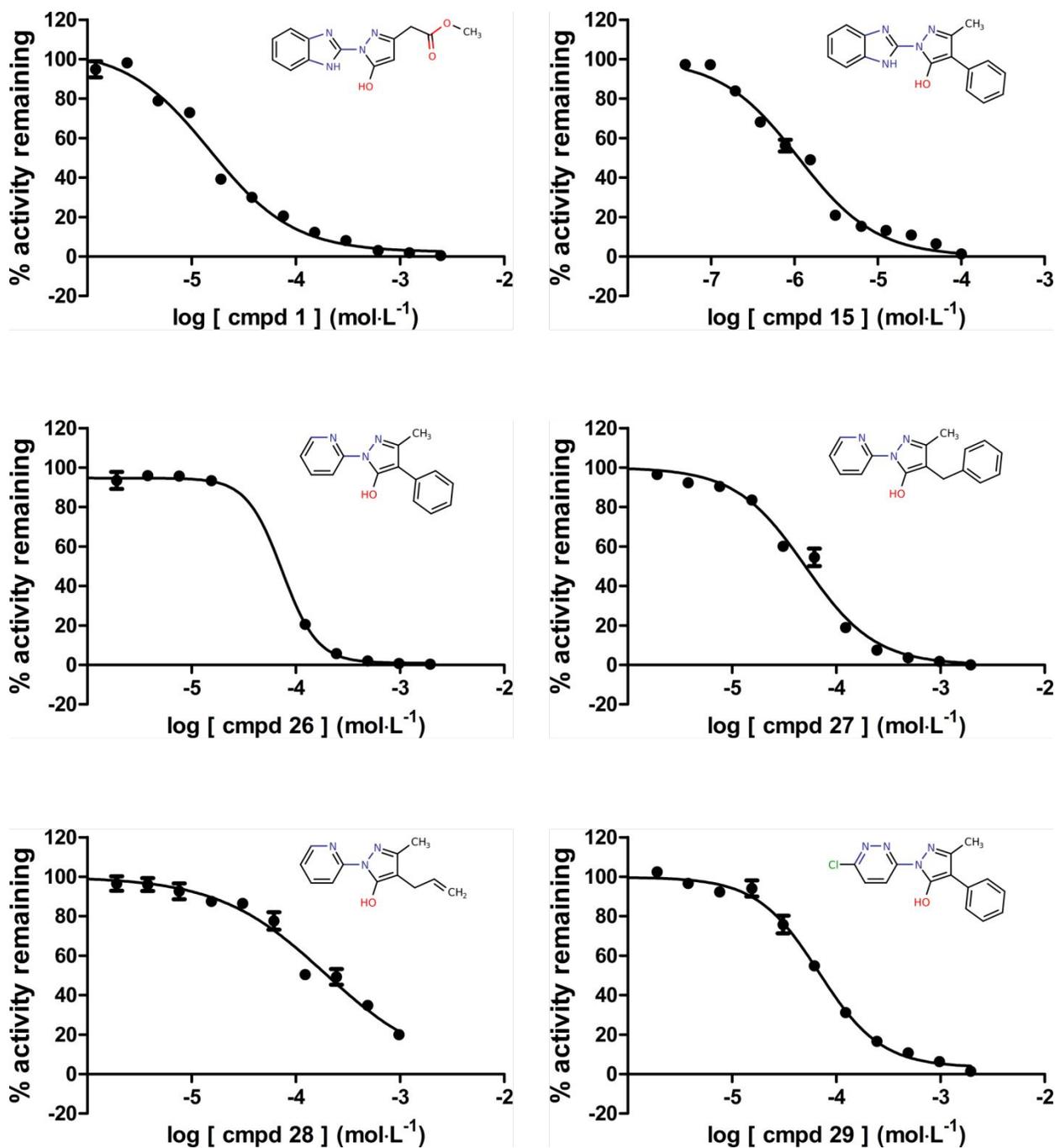
Figure S7. Inhibitory properties of selected KDM4 inhibitors, designed to assess the contribution of the scaffold's benzimidazole function towards inhibitory efficacy. Inhibition profiles resulting from FDH-based, KDM4E activity assays are depicted with best fit lines indicated using the log(inhibitor) vs. response -- Variable slope inhibition model in GraphPad Prism. The corresponding compound structures are depicted within each panel. Inhibitory profiles were assessed relative to data measured from the original HTS hit (compound **1**, top left) and the most potent inhibitor tested (compound **15**, top right).
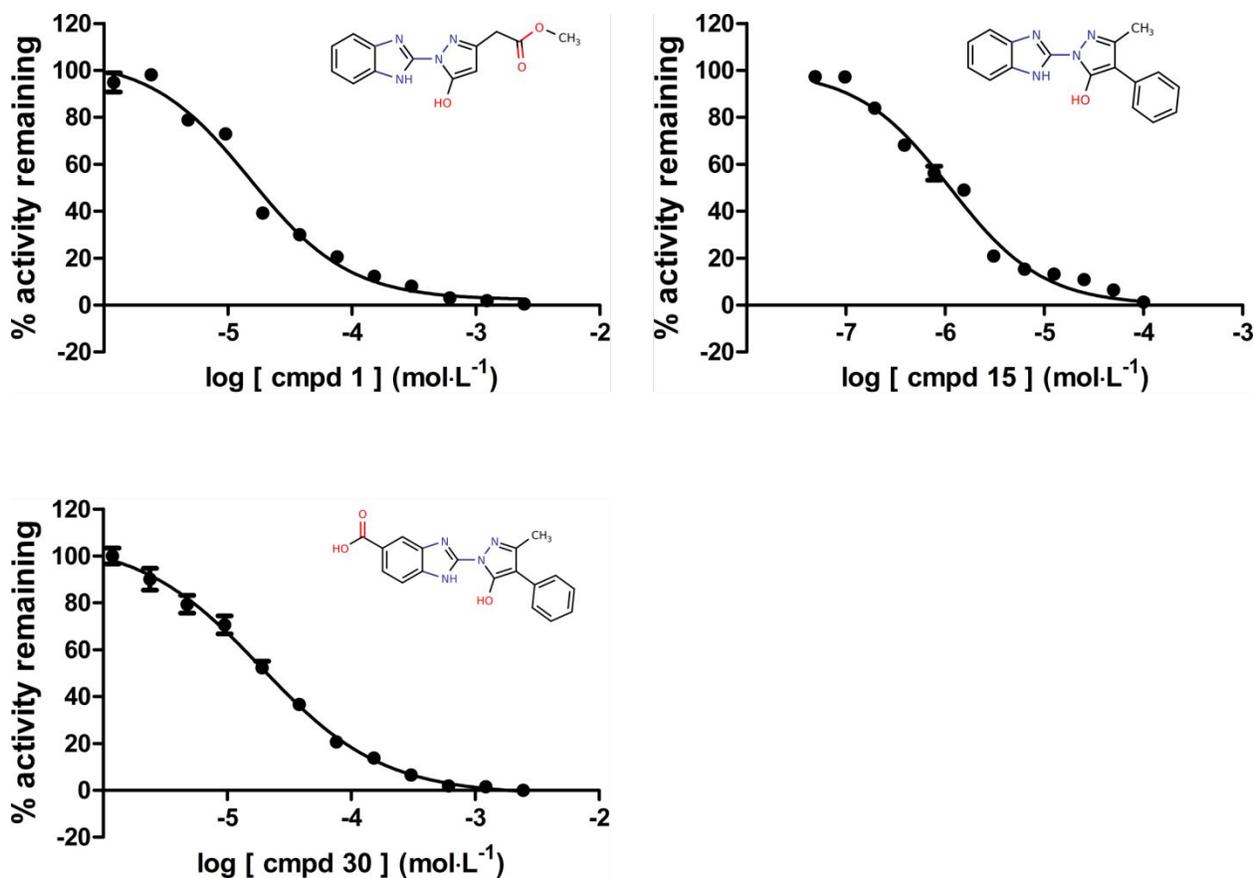
Figure S8. Inhibitory properties of selected KDM4 inhibitors, designed to assess the possibility to derivatize the scaffold's benzimidazole function. Inhibition profiles resulting from FDH-based, KDM4E activity assays are depicted with best fit lines indicated using the log(inhibitor) vs. response -- Variable slope inhibition model in GraphPad Prism. The corresponding compound structures are depicted within each panel. The inhibitory profile of **30** was assessed relative to data measured from the original HTS hit (compound **1**, top left) and the most potent inhibitor tested (compound **15**, top right).
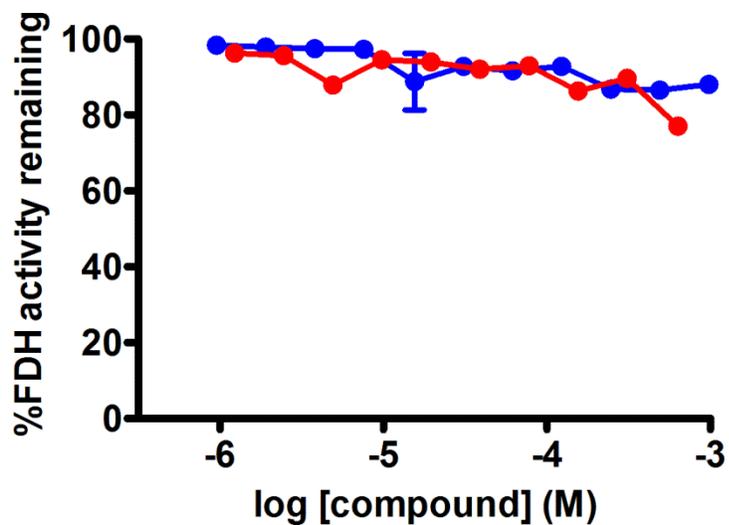
Figure S9. Enhanced properties of a benzimidazole benzylpyrazole KDM4E inhibitor do not affect activity of the coupling enzyme, formaldehyde dehydrogenase. The activity of FDH is plotted above as a function of KDM4 inhibitor concentration. Data points in blue reflect the original HTS hit (compound **1** in the main text). Data points in red reflect the most potent KDM4 inhibitor tested (compound **15** in the main text).

DU145 Cells
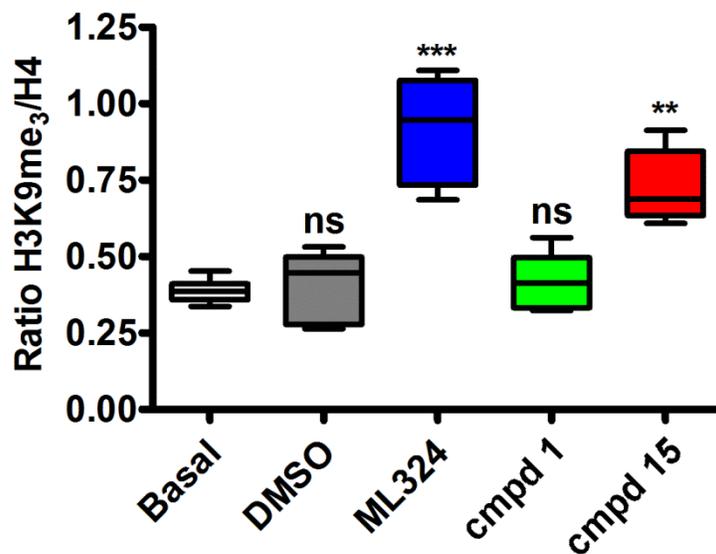
Figure S10. Compound **15** is active in cellular models of prostate cancer. Nucleosomal preparations from DU145 cells treated with membrane-permeable KDM4 inhibitors (ML324, blue; compound **15,** red) exhibit significantly higher levels of the H3K9me$_3$ epigenetic mark (normalized to a static histone H4 epitope), compared to untreated cells (white, labeled Basal), or to cells treated either with the non-permeable KDM4 inhibitor compound **1** (green), or with DMSO alone (grey). Statistical P values from t-tests were calculated relative to the signal arising from cells grown under basal conditions (for DMSO, ns = not significant), or relative to the signal arising from cells grown in the presence of DMSO (for ML324, ***P<0.0001; for compound **1**, ns = not significant; for compound **15**, **P=0.0016).