Supporting Information

Discovery and Mechanism Study of SIRT1 Activators that Promote the Deacetylation of Fluorophore-Labeled Substrate

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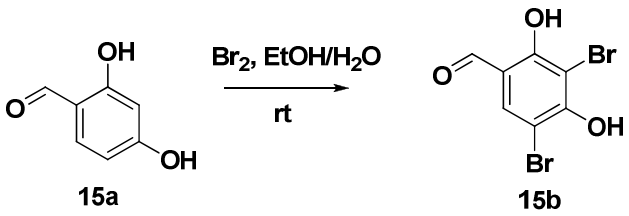
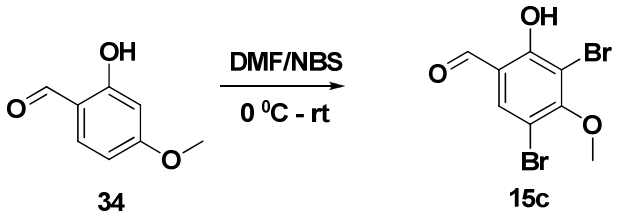
1. Chemistry

1.1. 3,5-Dibromo-2,4-dihydroxybenzaldehyde (15b).[1]



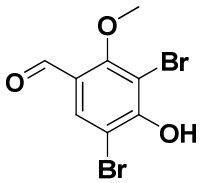
To a solution of 2,4-dihydroxybenzaldehyde (5 g, 36.20 mmol) in 50 mL of 95% EtOH was added dropwise Br_2 (4.64 mL, 90 mmol) at room temperature. The resulting mixture was stirred for 1 h. The precipitate was filtered, washed with $EtOH/H_2O$ (1/1, v/v) and dried to afford 6-bromonaphthalene-2-carbohydrazide as a light orange solid in 79% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.98 (s, 1H), 9.68 (s, 1H), 7.70 (s, 1H), 6.60 (s, 1H); MS (ESI, m/z): 292.8 $[M-H]^-$.

1.2. 3,5-Dibromo-2-hydroxy-4-methoxybenzaldehyde (15c).[2]



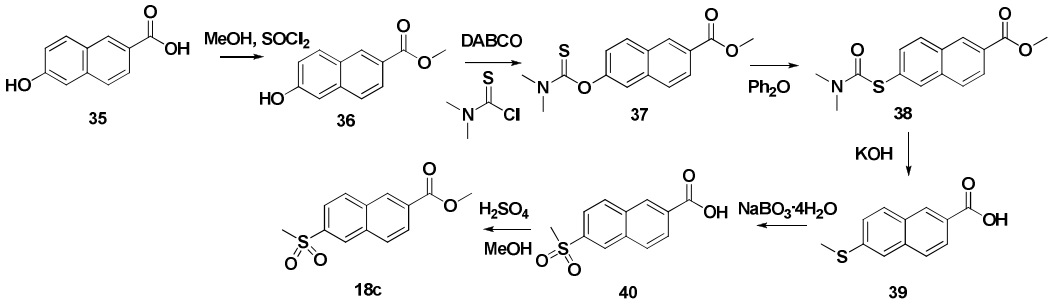
To an ice-cooled solution of 2-hydroxy-4-methoxybenzaldehyde (500 mg, 3.29 mmol) in 2 mL of DMF was added dropwise a solution of NBS (1.29 g, 7.23 mmol) in 2 mL of DMF. The resulting solution was stirred for additional 15 min and diluted with Et_2O (100 mL). The organic phase was successively washed with water (3 x 20 mL), saturated $Na_2S_2O_3$ (30 mL), dried over anhydrous Na_2SO_4 and concentrated under vacuum. The residue was recrystallized from hex/Et_2O to afford 3,5-dibromo-2-hydroxy-4-methoxybenzaldehyde (628 mg, 62 yield%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.01 (s, 1H), 7.88 (s, 1H), 3.90 (s, 3H); MS (ESI, m/z): 306.8 $[M-H]^-$.

1.3. 3,5-Dibromo-4-hydroxy-2-methoxybenzaldehyde (15d).



Prepared in a similar manner as described for compound **15c**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.01 (s, 1H), 7.88 (s, 1H), 3.90 (s, 3H).

1.4. Methyl 6-(methylsulfonyl)-2-naphthoate (18c).[3]



To a solution of 6-hydroxy-2-naphthoic acid **35** (500 mg, 2.66 mmol) in 5 mL of MeOH was added slowly thionyl chloride (0.48 mL, 6.64 mmol). The resulting solution was refluxed for 3 h. After concentration, the residue was diluted EA and washed with saturated $NaHO_3$. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under vacuum to afford methyl 6-hydroxy-2-naphthoate **36** (511 mg, 95% yield) as a white solid, which was used in the next step without further purification. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.18 (s, 1H), 8.50 (s, 1H), 7.98 (d, J = 8.4 Hz, 1H), 7.86 (dd, J = 1.6, 8.0 Hz, 1H), 7.77 (d, J = 9.0 Hz, 1H), 7.21-7.15 (m, 2H), 3.88 (s, 3H).

A mixture of methyl 6-hydroxy-2-naphthoate **36** (450 mg, 2.23 mmol), dimethylcarbamothioic chloride (344 mg, 2.78 mmol) and DABCO (312 mg, 2.78 mmol) in 6 mL of DMF was stirred at room temperature overnight. The reaction solution was diluted with water (60 mL). The mixture was extracted with EA (20 mL x 3). The combined organic layer was washed with waterand brine, dried over anhydrous Na_2SO_4 and concentrated to afford methyl 6-(dimethylcarbamothioyloxy)-2-naphthoate **37** (625 mg) as a light yellow solid, which was used in the next step. H NMR (300 MHz, DMSO-d_6) δ 8.66 (s, 1H), 8.17 (d, J = 9.0 Hz, 1H), 8.05-7.98 (m, 2H), 7.71 (d, J = 2.1 Hz, 1H), 7.38 (dd, J = 2.1, 8.7 Hz, 1H), 3.93 (s, 3H), 3.40 (s, 3H), 3.38 (s, 3H).

A suspension of methyl 6-(dimethylcarbamothioyloxy)-2-naphthoate **37** (625 mg, 2.16 mmol) in 6 mL of Ph_2O was heated at 200 ^{0}C for 4 h under argon inert atmosphere. After cooled to room temperature, the mixture was purified by silica gel column chromatography (PE/EA 1/6-4/1, v/v) to afford methyl 6-(dimethylcarbamoylthio)-2-naphthoate **38** (186 mg, 29.7%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.67 (s, 1H), 8.19-8.13 (m, 2H), 8.11-8.00 (m, 2H), 7.59 (dd, J = 1.8, 8.7 Hz, 1H), 3.93 (s, 3H), 3.09 (s, 3H), 2.95 (s, 3H).

To a suspension of 6-(dimethylcarbamoylthio)-2-naphthoate **38** (182 mg, 0.63 mmol) in 6 mL of MeOH was added 10 mL of 5N KOH. The mixture was refluxed for 3 h and cooled with ice-water bath. Dimethyl sulfate (0.66 mL, 8.04 mmol) was added dropwise to the mixture. The resulting mixture was warmed to room temperature and stirred for additional 4 h and acidified to PH ~3 using concentrated HCl. The white precipitate was collected by filtration and dried to afford 6-(methylthio)-2-naphthoic acid **39** (132 mg, 94% yield) as a white solid. MS (ESI, m/z): 217.0 $[M-H]^-$.

A suspension of 6-(methylthio)-2-naphthoic acid **39** (150 mg, 0.69 mmol) and $NaBO_3 \cdot 4H_2O$ (899 mg, 5.84 mmol) in 4 mL of AcOH was heated at 55 ^{0}C for 2 h. Water was added to the suspension and white solid was precipitated. The white solid was collected by filtration and dried to afford 6-(methylsulfonyl)-2-naphthoic acid **40** (77 mg, 45% yield). MS (ESI, m/z): 249.0 $[M-H]^-$.

To a suspension of 6-(methylsulfonyl)-2-naphthoic acid **40** in 4 mL of MeOH was added catalytic amount of concentrated H_2SO_4. The resulting mixture was refluxed overnight. After the removal of organic solvent, the residue was extracted with chloroform. The organic layer was washed with saturated $NaHCO_3$, dried over anhydrous sodium sulfate and concentrated under vacuum to afford methyl 6-(methylsulfonyl)-2-naphthoate **18c**, which was used in the next step without further purification. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.79 (s, 1H), 8.69 (d, J = 1.5 Hz, 1H), 8.42 (d, J = 8.7 Hz, 1H), 8.33 (d, J = 8.4 Hz, 1H), 8.13 (dd, J = 1.5, 8.7 Hz, 1H), 8.05 (dd, J = 1.8, 8.7 Hz, 1H), 3.94 (s, 3H), 3.33 (s, 3H).

1.5. HPLC Analysis Data of the Diaryl Acylhydrozone Analogues

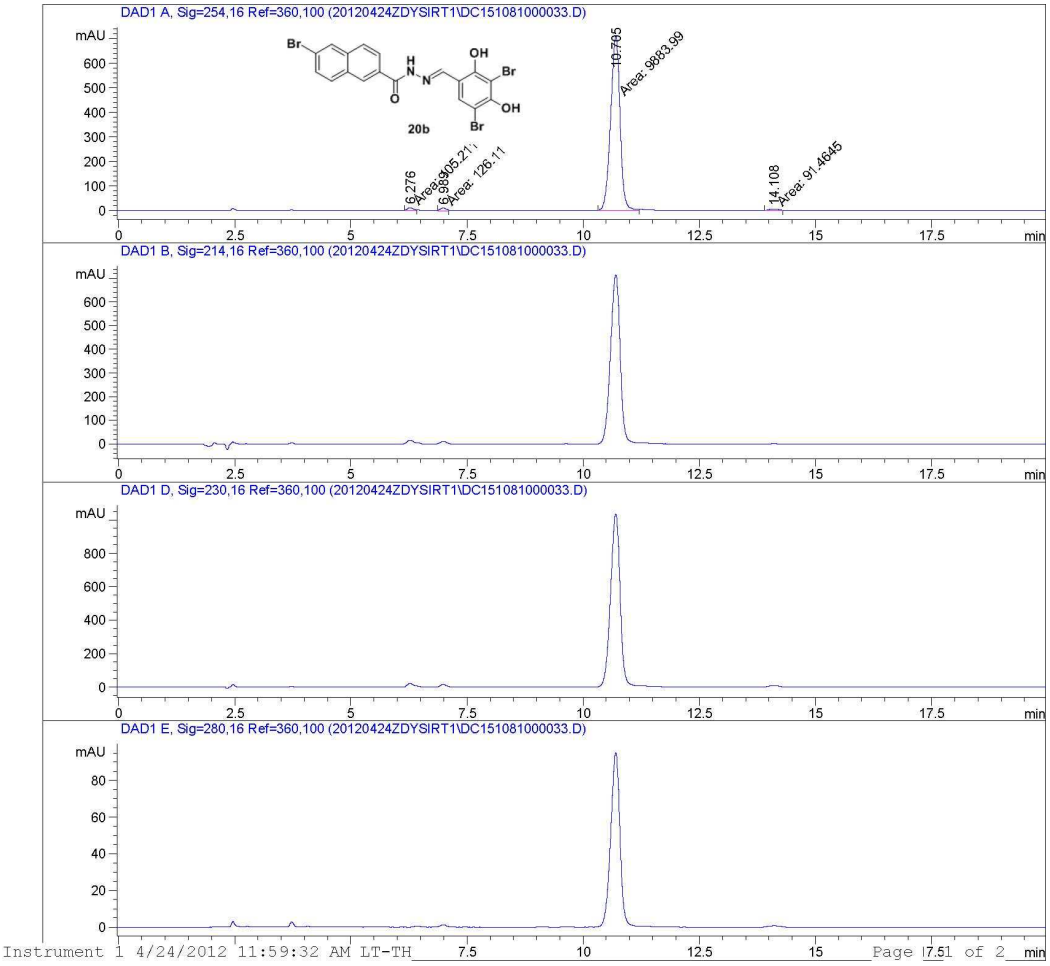
1.5.1. General method for HPLC analysis: HPLC analysis was conducted according to different eluent. The retention time (t_R) is expressed in min at UV detection. HPLC analysis was performed on a Zorbax Eclipse XBD-C18 column (4.6 × 150 mm, 5 mm) at 23 ℃.

Compounds	Retention Time (Min)	Relative Purity (%)
16a	10.18 [a]	99.28
16b	8.85 [b]	99.03
16c	6.89 [a]	96.09
16d	23.56 [b]	95.70
16e	6.43 [c]	98.13
16f	16.59 [a]	99.29
16g	6.32 [a]	96.32
16h	14.51 [d]	97.26
16i	2.27 [a]	95.71
16j	3.32 [b]	98.59
16k	3.90 [b]	99.03
16l	9.18 [b]	98.90

16m	13.82 [b]	98.94
16n	9.41 [b]	98.41
20a	3.59 [b]	97.17
20b	10.71 [a]	96.84
20c	8.04 [b]	97.64
20d	8.59 [a]	95.16
20e	5.64 [b]	96.63
20f	10.50 [b]	95.79
20g	16.07 [b]	95.11
20h	10.79 [e]	98.20
20i	15.38 [a]	97.09

[a] CH_3CN(0.1% TFA)/H_2O (0.1% TFA) (65/35, v/v, flow rate: 0.6 mL/Min, 254 nM); [b] CH_3OH(0.1% TFA)/H_2O (0.1% TFA) (70/30, v/v, flow rate: 0.6 mL/Min, 254 nM); [c] CH_3OH/H_2O (70/30, v/v, flow rate: 0.8 mL/Min, 254 nM); [d] CH_3OH(0.1% TFA)/H_2O (0.1% TFA) (70/30, v/v, flow rate: 0.6 mL/Min, 230 nM); [e] CH_3CN(0.1% TFA)/H_2O (0.1% TFA) (65/35, v/v, flow rate: 0.6 mL/Min, 230 nM).

1.5.2. HPLC spectra of compound 20b



Signal 1: DAD1 A, Sig=254,16 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	6.276	MM	0.1538	105.21089	11.40153	1.0308
2	6.989	MM	0.1648	126.11032	12.75508	1.2356
3	10.705	MM	0.2318	9883.98828	710.68201	96.8375
4	14.108	MM	0.2651	91.46450	5.75045	0.8961
Totals :				1.02068e4	740.58907	

Signal 2: DAD1 B, Sig=214,16 Ref=360,100

Signal 3: DAD1 D, Sig=230,16 Ref=360,100

Signal 4: DAD1 E, Sig=280,16 Ref=360,100

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2. Biology

Table S1. The activiation of SIRT1, SIRT2 and SIRT3 by the compounds at the concentration of 50 μM. The data were obtained by at least three independent experiments. Reaction wells with no compound were used as control. The signal from the sample wells was divided by the signal from the control wells and then multiplies by 100 to give the "percentage of control".

Compound	Percentage of Control		
	SIRT1	SIRT2	SIRT3
1	197±13 [a]	114±5	105±1
7	217±12	126±9	102±2
8	192±22	187±3	102±1
9	173±4	85±1	86±1
10	375±6	109±10	89±1
11	325±2	83±16	81±2
16a	170±10	121±6	121±1
16b	184±3	87±2	78±2
16c	339±18	119±3	93±3
16d	209±2	103±5	98±3
16e	131±1	85±4	86±3
16f	246±5	95±1	90±4
16g	186±3	88±3	98±3
16h	263±16	91±1	88±1
16i	317±17	86±3	84±3
16j	292±11	82±12	88±3
16k	288±4	84±1	98±8
16l	137±4	85±1	84±1
16m	130±6	89±7	93±1
16n	109±1	110±3	101±1
20a	146±5	125±14	88±2

20b	649±10	90±1	105±3
20c	114±14	117±3	92±5
20d	233±11	92±4	88±3
20e	364±20	92±2	89±1
20f	421±5	89±3	85±3
20g	609±13	89±7	83±1
20h	217±7	118±17	103±3
20i	191±3	200±7	183±4

[a] 197±13: 197 means that compound **1** activates SIRT1 by 1.97-fold at the indicated concentration; 13 represents the standard deviation of three independent experiments.

Figure S1. Isothermal titration calorimetry was performed to detect the binding ability of peptide to SIRT1. The data was fit to a one-site binding model. **A)** 2.5 mM ac-RHKK$_{ac}$-NH$_2$ was titrated to 102 μM SIRT1. The K_d value is 44.4±11.0 μM (Mean±SD, for three independent measurements). **B)** 2.5 mM ac-RHKK$_{ac}$-AMC was titrated to 124 μM SIRT1. The K_d value is 184.3±9.8 μM (Mean±SD, for two independent measurements).

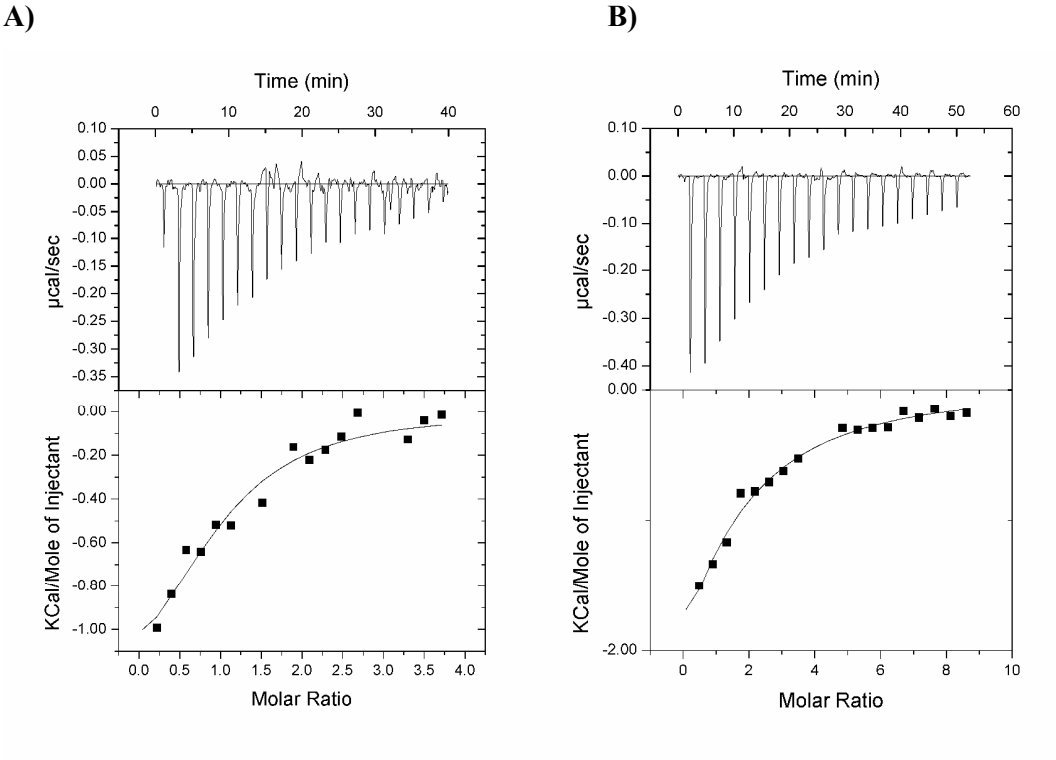
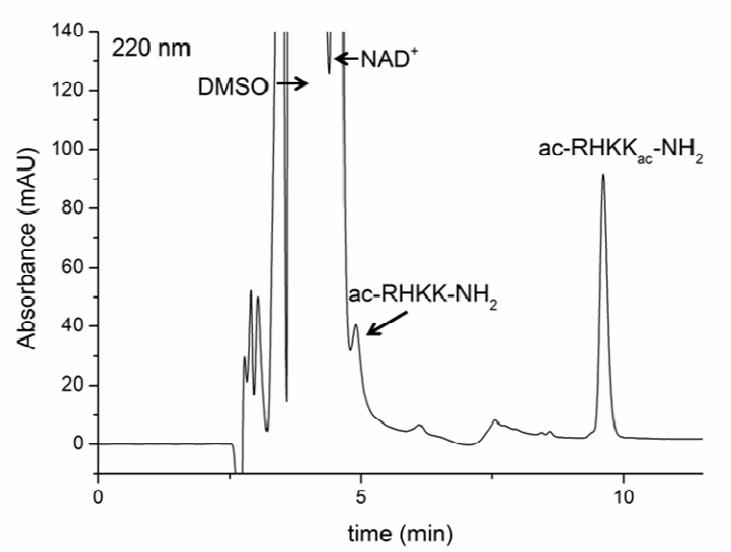


Figure S2. HPLC chromatography was used to quantify the deacetylation of AMC-labeled or unlabeled peptide. **A)** The absorbance curve (220 nm) of fluorophore-unlabeled substrate, ac-RHKK$_{ac}$-NH$_2$. The peaks corresponding to DMSO, NAD$^+$ and ac-RHKK-NH$_2$ were indicated with arrows. **B)** The absorbance curve (325 nm) of AMC-labeled substrate, ac-RHKK$_{ac}$-AMC.

A)



B)

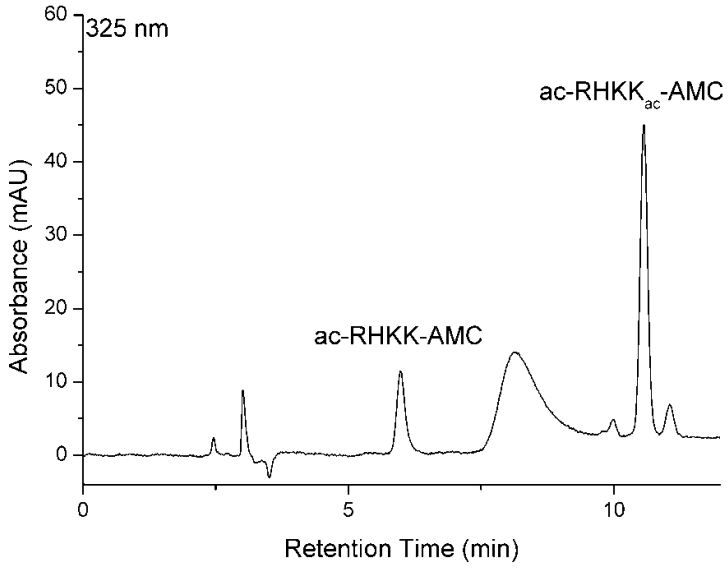


Figure S3. The effect of the indicated compounds on SIRT1 deacetylation activity. White columns represent the reaction with ac-RHKK$_{ac}$-AMC as substrate; grey columns represent the reaction with ac-RHKK$_{ac}$-NH$_2$ as substrate. The reaction was performed under the same condition as the screening assay and detected by HPLC as described in the experimental section.

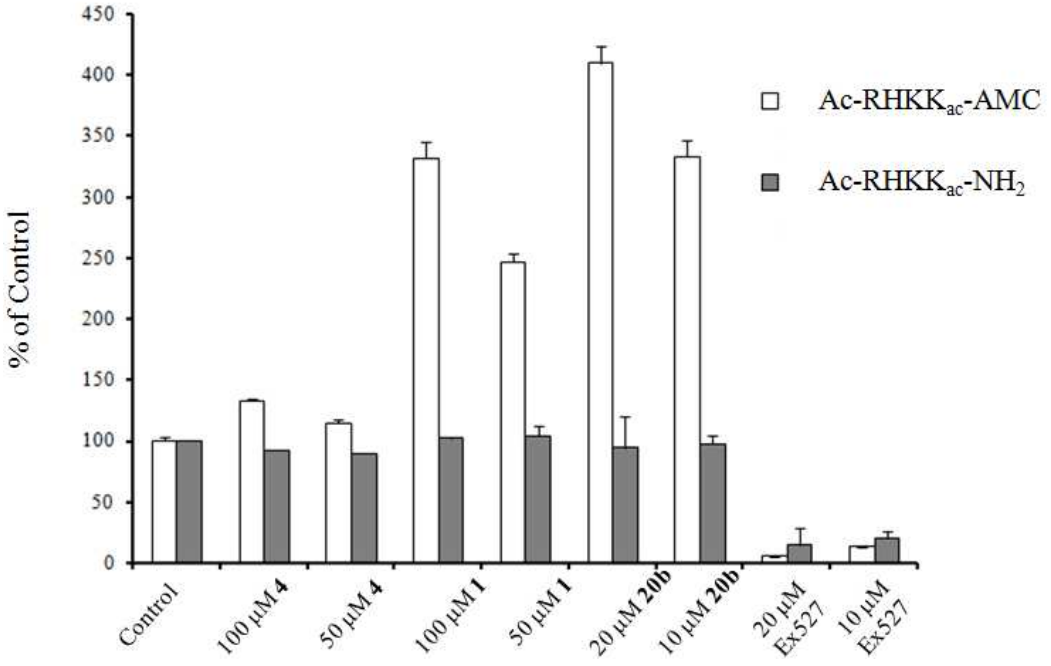


Figure S4. Sequence alignment of SIRT1 and SIRT3.

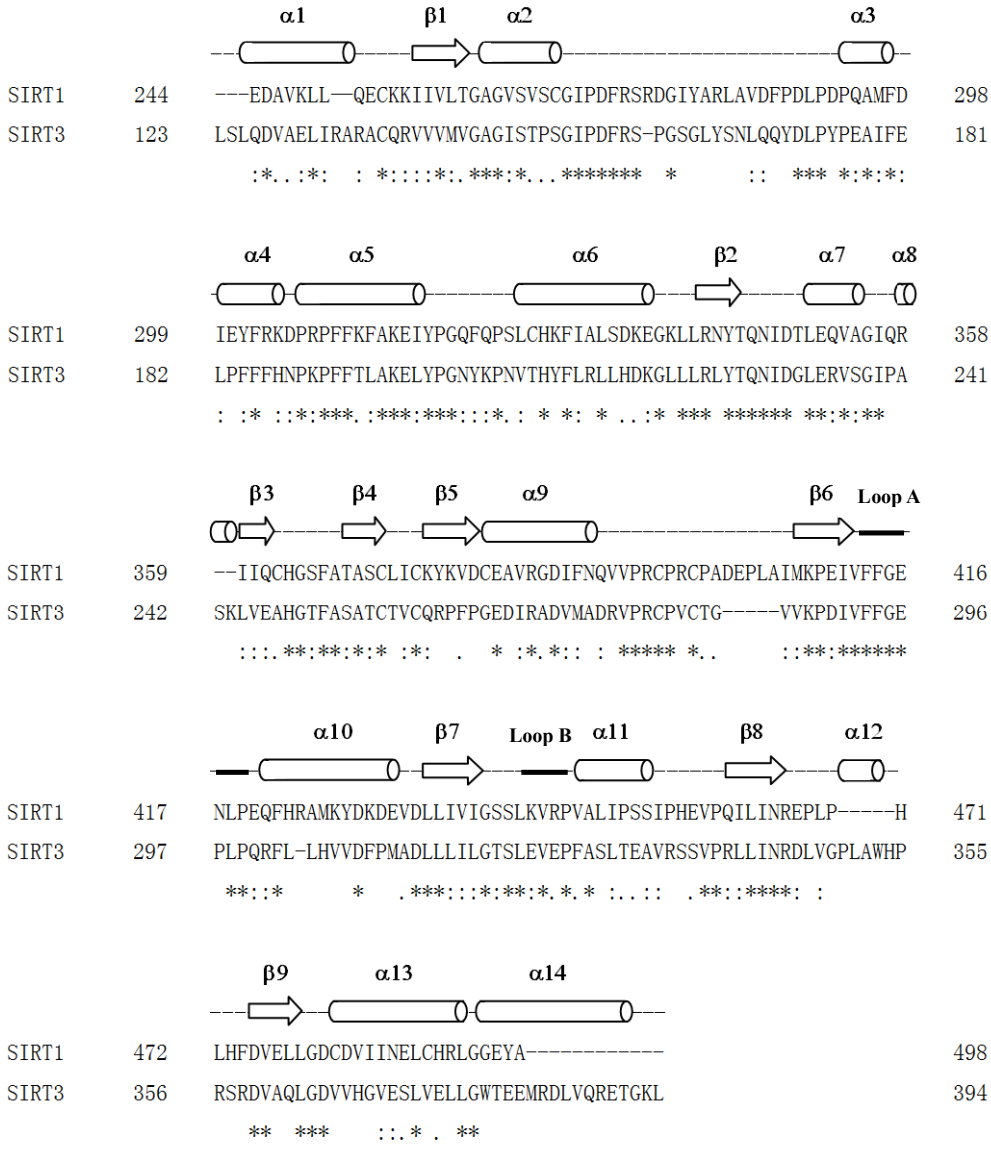
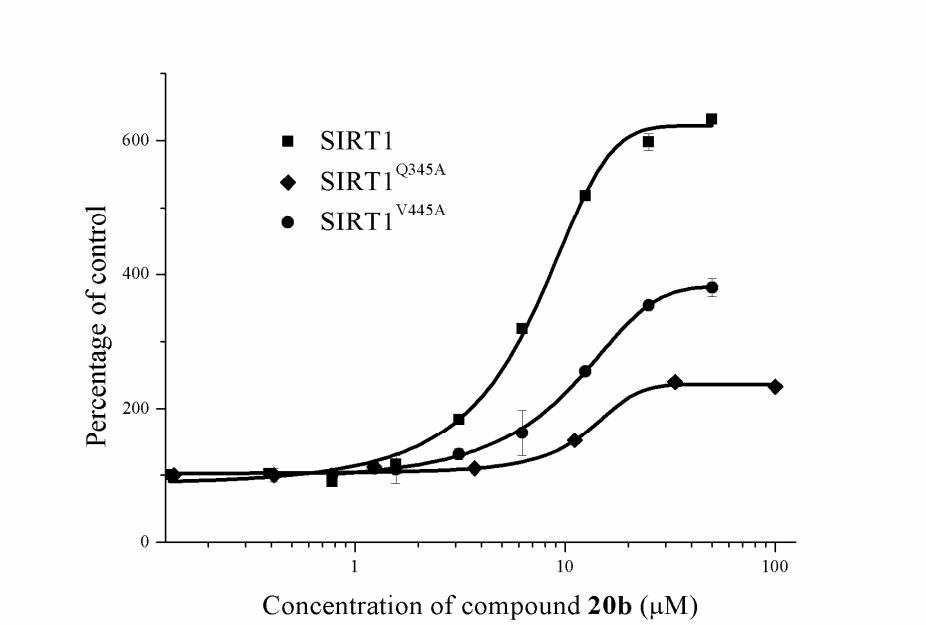


Figure S5. A) Dose-response curves of **20b** against SIRT1 wild type and mutants $SIRT1^{Q345A}$ and $SIRT1^{V445A}$. **B)** Lineweaver-burk plots of ac-$RHKK_{ac}$-AMC peptide for SIRT1 wild type and mutants in the absence and presence of 10 μM of **20b**.

A)



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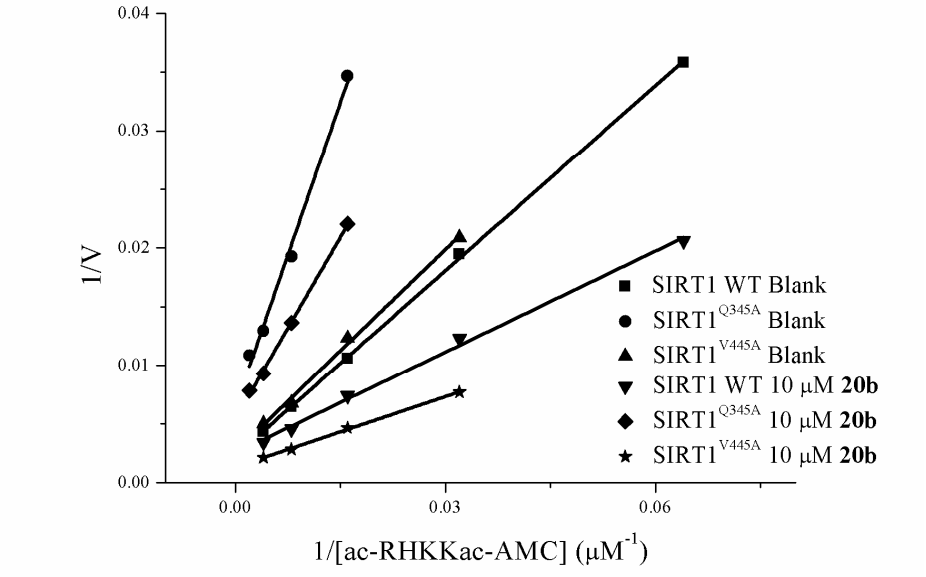
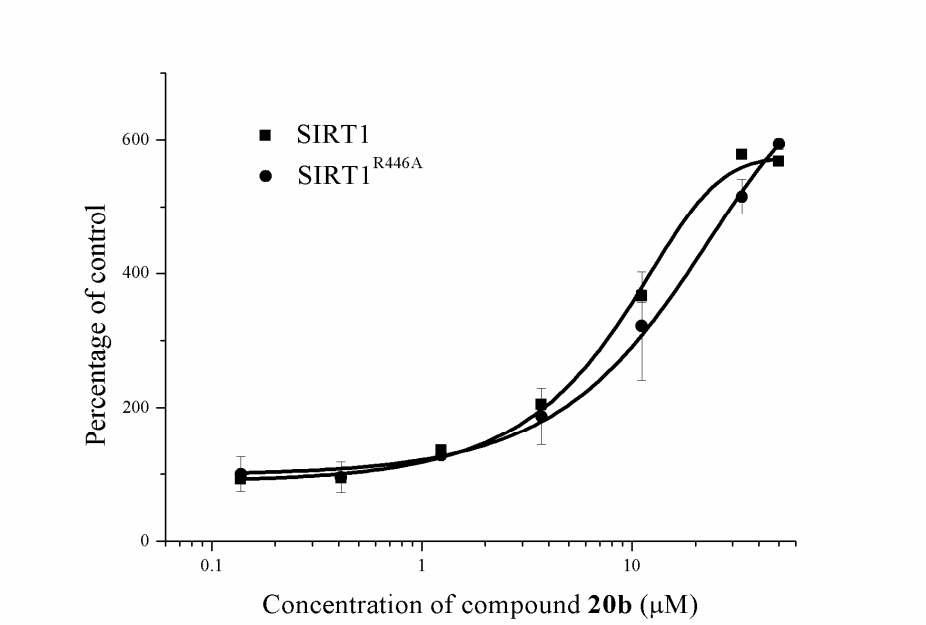


Figure S6. A) Dose-response curves of **20b** against SIRT1 wild type and mutant $SIRT1^{R446A}$. **B)** Dose-response curves of **1** against SIRT1 wild type and mutant $SIRT1^{R446A}$. **C)** Lineweaver-burk plots of ac-RHKK$_{ac}$-AMC peptide for SIRT1 wild type and $SIRT1^{R446A}$ in the absence and presence of 50 μM of **1**.

A)



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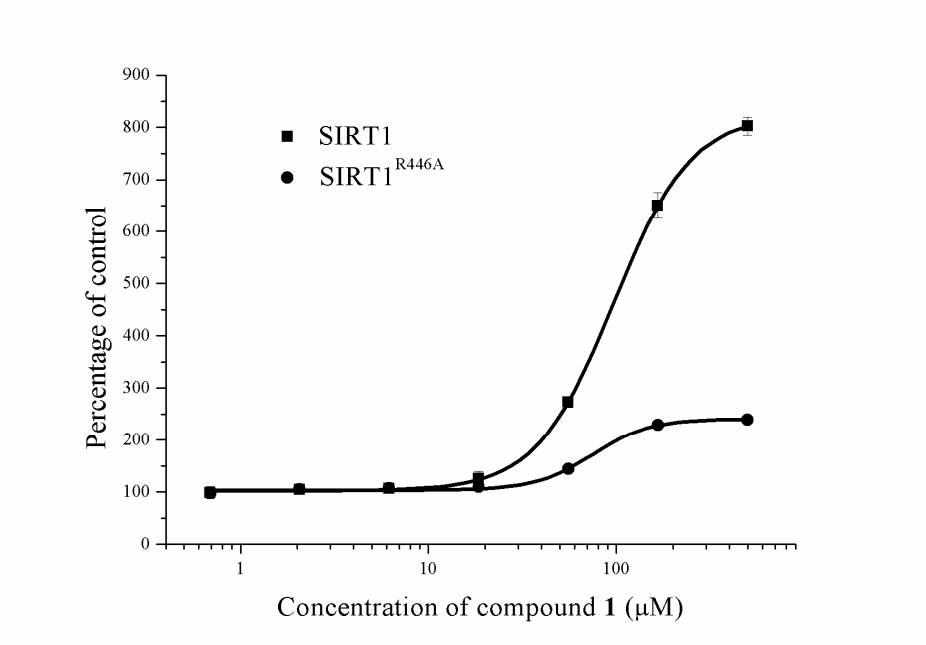


C)

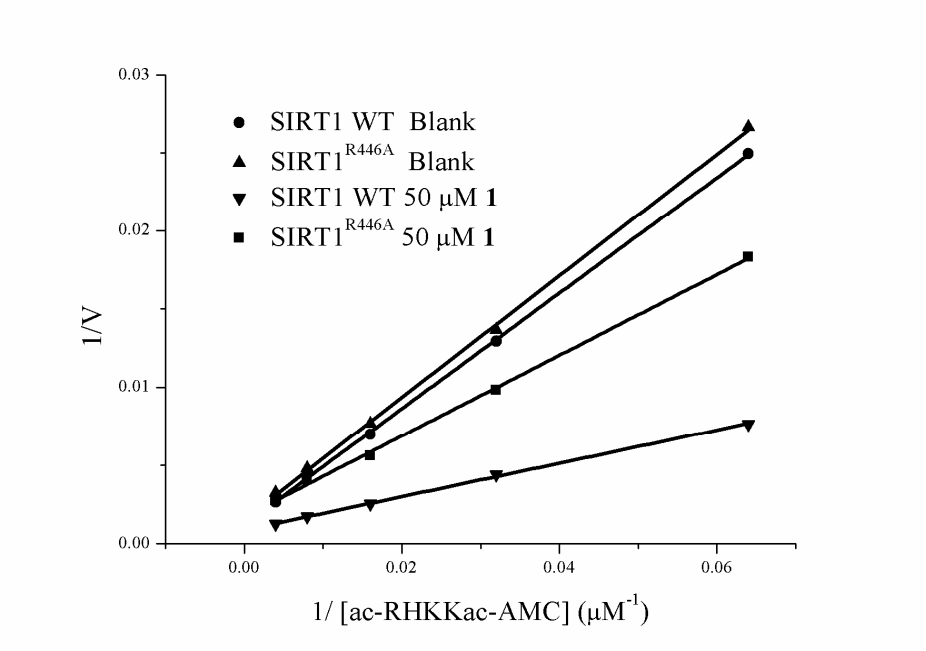
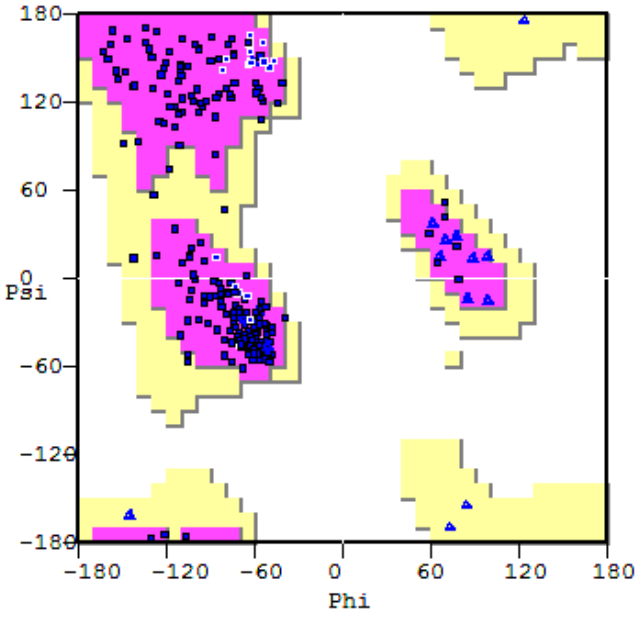

0.03
0.02
0.01
0.00
1/V
SIRT1 WT Blank
SIRT1R446A Blank
SIRT1 WT 50 μM 1
SIRT1R446A 50 μM 1
0.00
0.02
0.04
0.06
1/ [ac-RHKKac-AMC] (μM-1)

Figure S7. A) Ramanchandran plot of "SIRT3/ac-RHKK$_{ac}$-AMC" complex crystal structrure. The statistic result of the plot was listed in Table 4. **B)** Ramanchandran plot of "SIRT1/ac-RHKK$_{ac}$-AMC" complex model structure. The plot revealed that 95.33 % residues are in the preferred region, 3.89 % residues in the allowed region, and 0.78 % residues in the outlier.

A)



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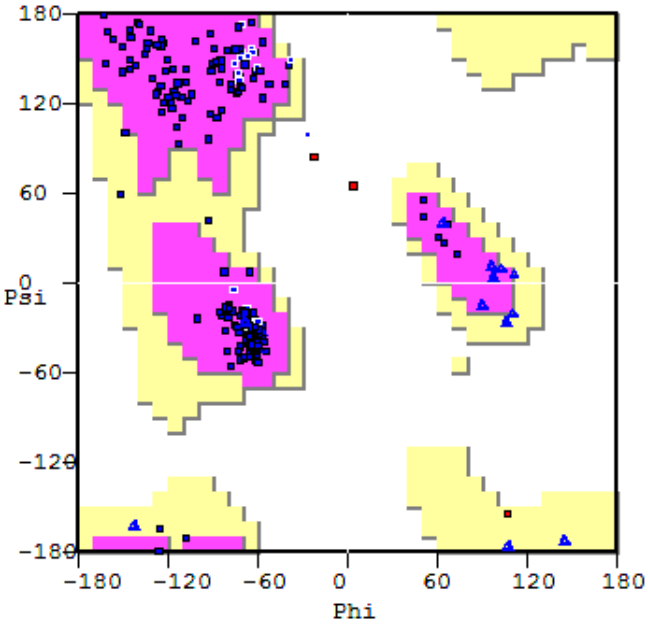

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