Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

Multifunctional Activity-based Chemical Probes for Sirtuins

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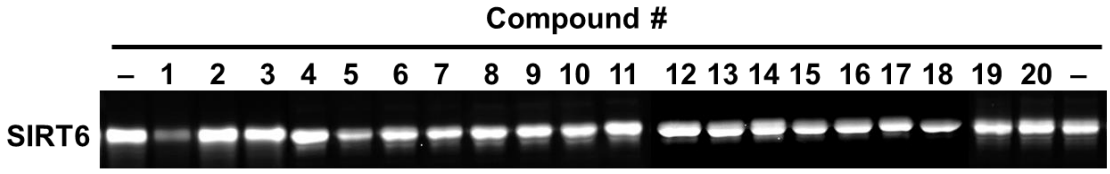
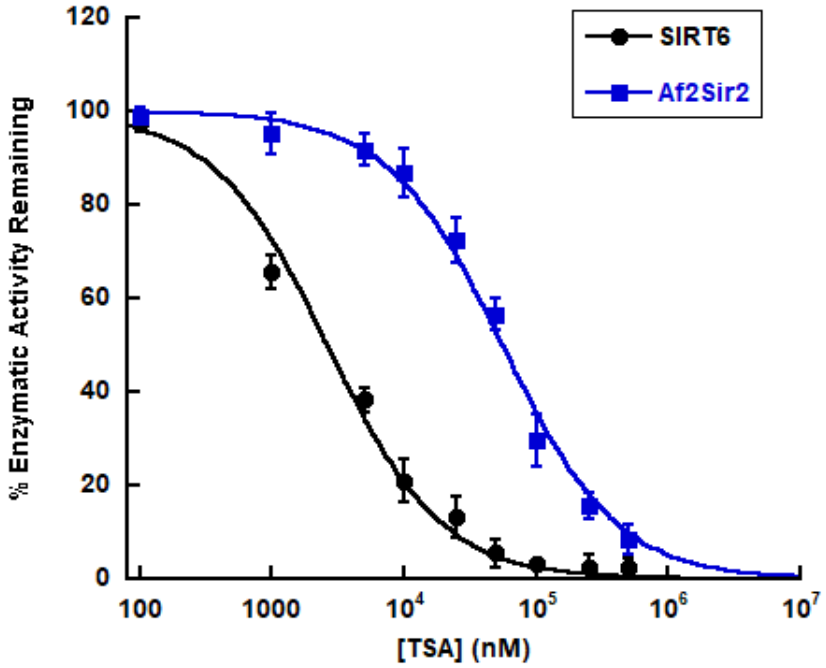
Figure S1. Inhibitor screening panel of 20 small molecules against SIRT6.



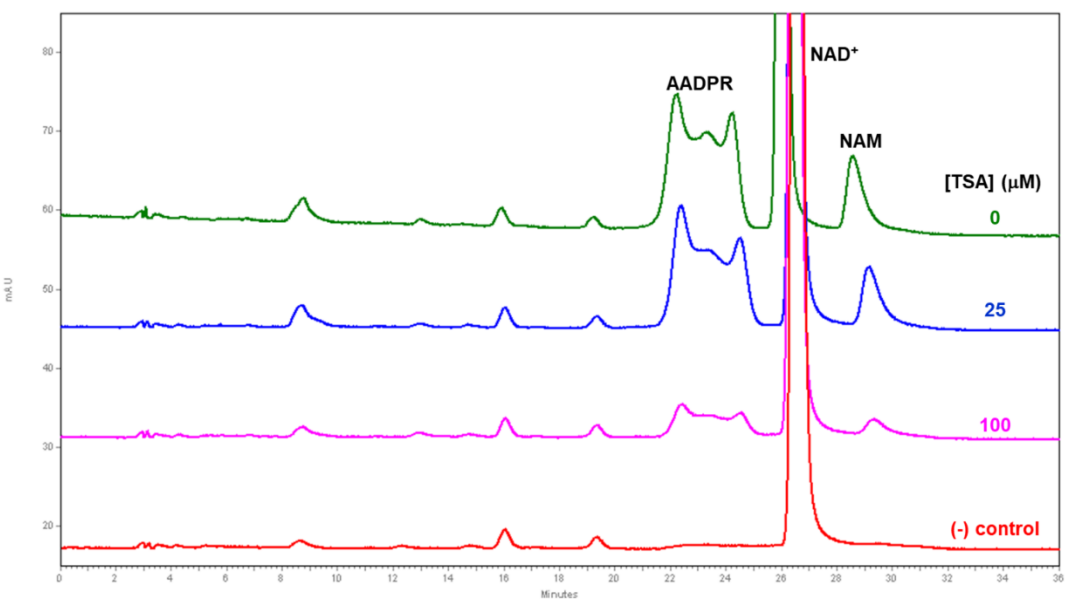
Representative gel images of SIRT6 inhibitor screening. SIRT6 was incubated with 100 μM candidate compound or vehicle (DMSO) in the presence of 500 μM NAD^+ for 30 min at 37°C. Subsequently, probe 1B was added to a final concentration of 25 μM and labeled as described in "Methods and Materials". The samples were resolved by SDS-PAGE and analyzed with in-gel fluorescence scanning using a Biorad ChemiDoc MP imager (excitation 532 nm, 580 nm cut-off filter and 30 nm band-pass).

Figure S2. Concentration-dependent inhibition of SIRT6 and *Af*2Sir2 by TSA.

A

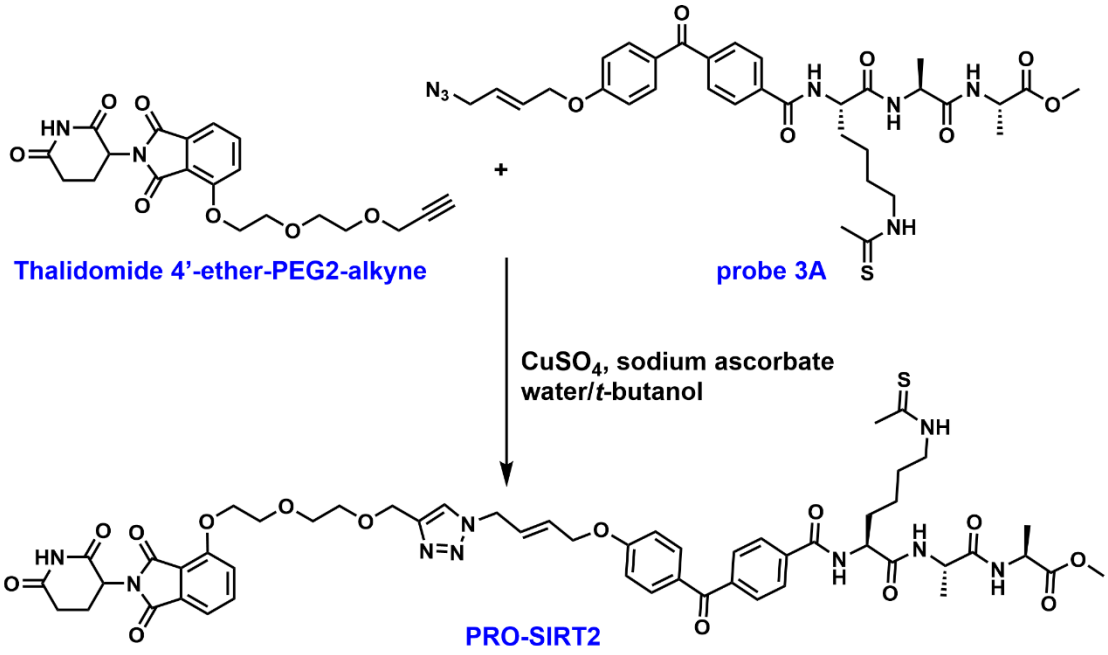


B



A. Dose-response curves for SIRT6 and *Af2*Sir2. The experimental details can be found in "Methods and Materials"; B. Representative HPLC chromatograms showing the inhibition of *Af*2Sir2 activity by TSA. The forklike peak represents AADPR (a mixture of 2'-AADPR and 3'-AADPR). *Af*2Sir2 was absent in the negative control.

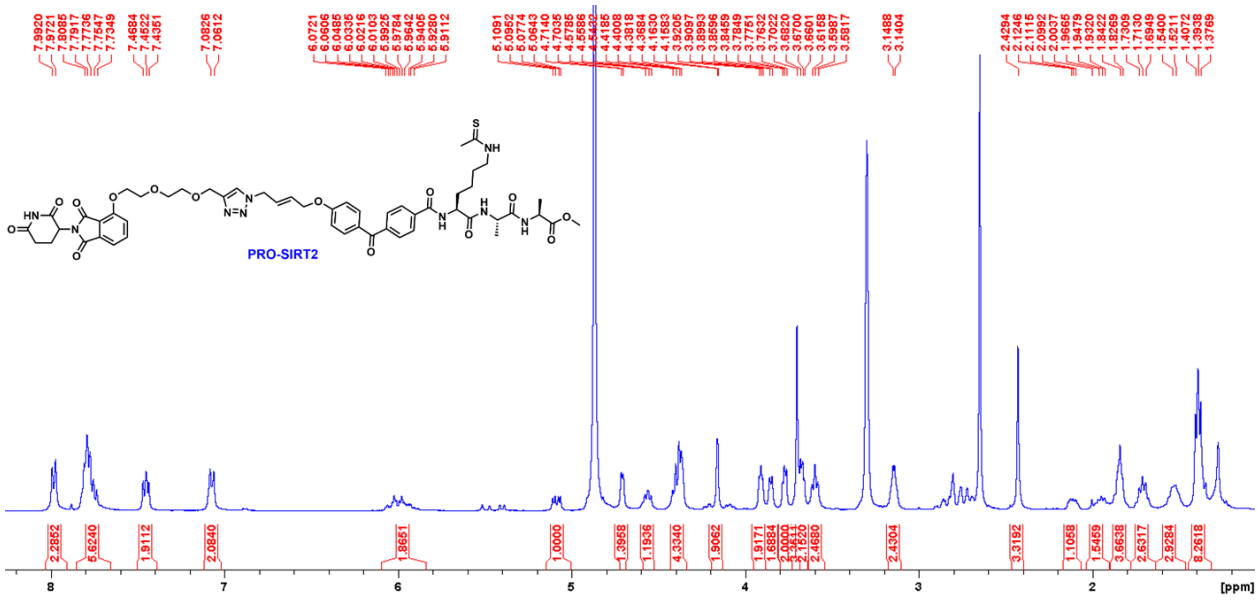
Synthesis of PRO-SIRT2

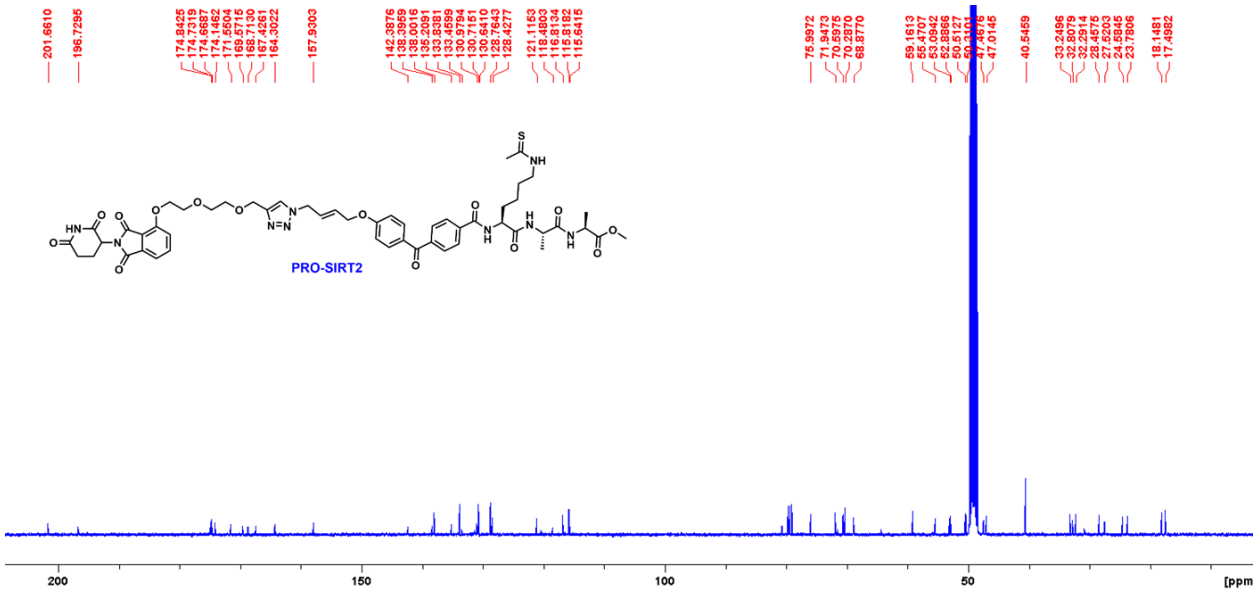


Synthesis of probe 3A. Probe 3A was synthesized as described previously.[1]

Synthesis of PRO-SIRT2. Probe 3A (17 mg, 0.025 mmol) and thalidomide 4'-ether-PEG2-alkyne (10 mg, 0.025 mmol) were dissolved in a mixture of water and *tert*-butanol (2 mL, v/v = 1/1). To this solution were added freshly prepared $CuSO_4$ solution (2.5 mM) and sodium ascorbate solution (5 mM). The reaction mixture was stirred at room temperature overnight. Reaction was quenched with water, aqueous layer was extracted with CH_2Cl_2, combined organic layer was washed with brine and dried over Na_2SO_4. Solvent was evaporated under reduced pressure, and the crude product was purified by silica gel column chromatography (CH_2Cl_2 with 10 to 20% methanol) to afford **PRO-SIRT2** (15 mg, 0.014 mmol, 55% yield) as a pale yellow oil. ^{1}H NMR (CD_3OD, 400 MHz) δ (ppm): 1.35 (m, 2H), 1.38 (d, J = 5.4 Hz, 3H), 1.40 (d, J = 5.4 Hz, 3H), 1.53 (m, 3H), 1.71 (m, 3H), 1.83 (m, 4H), 1.95 (m, 2H), 2.11 (m, 1H), 2.43 (s, 3H), 3.14 (m, 2H), 3.60 (t, J = 6.8 Hz, 2H), 3.68 (m, 2H), 3.70 (s, 3H), 3.78 (m, 2H), 3.85 (d, J = 5.5 Hz, 2H), 3.91 (m, 2H),

4.16 (d, *J* = 1.9 Hz, 2H), 4.39 (m, 4H), 4.56, (m, 1H), 4.70 (d, *J* = 4.2 Hz, 1H), 5.08 (dd, *J* = 5.6, 12.7 Hz, 1H), 6.01 (m, 2H), 7.07 (d, *J* = 8.6 Hz, 2H), 7.45 (t, *J* = 6.6 Hz, 2H), 7.78 (m, 6H), 7.98 (d, *J* = 8.0 Hz, 2H). ^{13}C NMR (CD_3OD, 100 MHz) δ (ppm): 17.5, 18.2, 23.8, 24.6, 27.5, 28.5, 32.3, 32.8, 33.3, 40.6, 47.0, 47.5, 50.3, 50.5, 52.9, 53.1, 55.5, 59.2, 68.9, 70.3, 70.6, 72.0, 76.0, 115.6, 115.8, 116.8, 118.5, 121.1, 128.4, 128.8, 130.6, 130.7, 131.0, 133.5, 133.8, 135.2, 138.0, 138.4, 142.4, 157.9, 164.3, 167.4, 168.7, 169.6, 171.6, 174.2, 174.7, 174.7, 174.9, 196.7, 201.7. HRMS (*m/z*): calculated for $C_{53}H_{62}N_9O_{14}S$ (M+H): 1080.4131; found: 1080.4135.


PRO-SIRT2
[ppm]


PRO-SIRT2
[ppm]

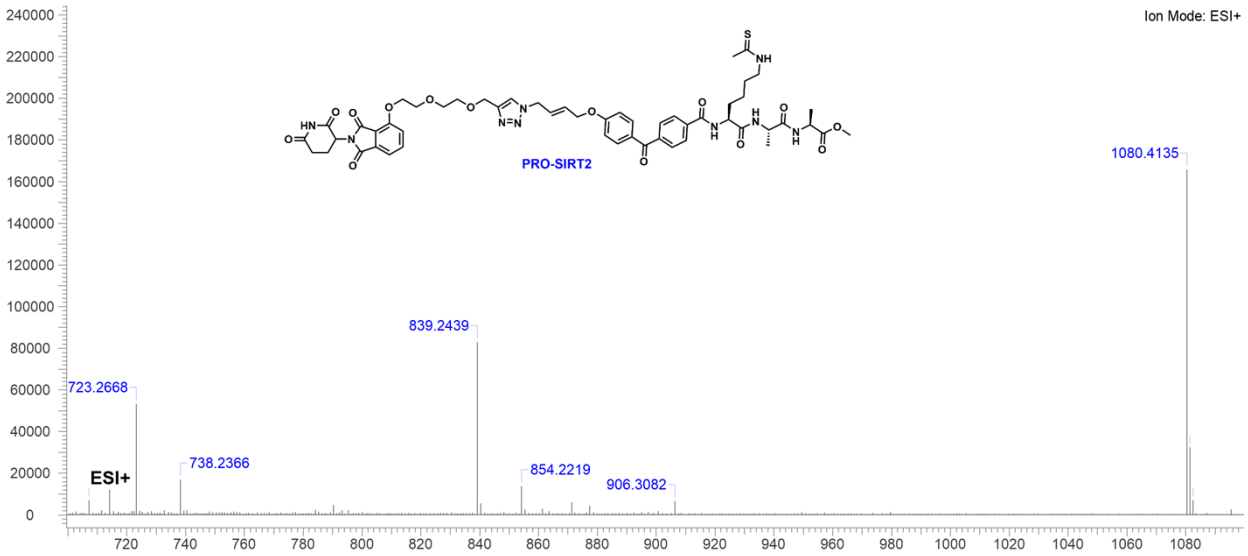
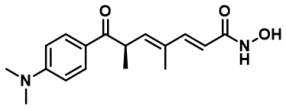
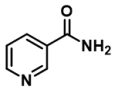
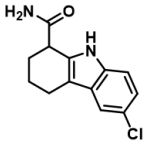
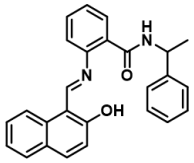
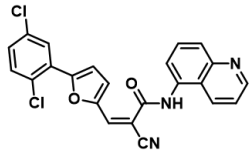
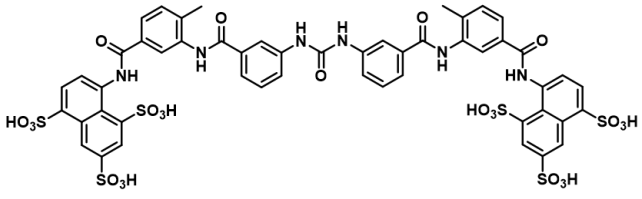
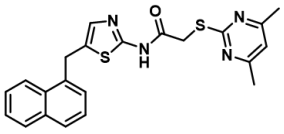
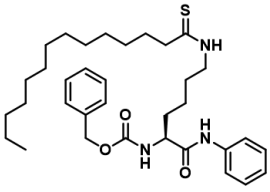
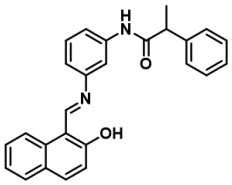
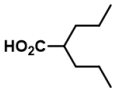
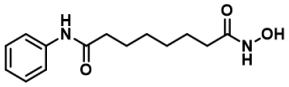
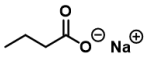
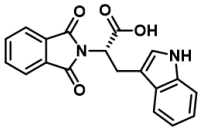
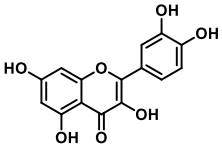
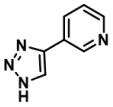
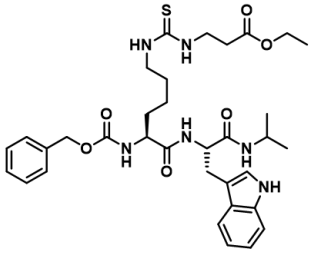
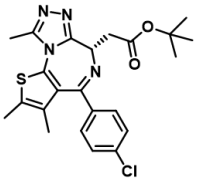
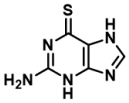
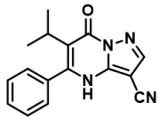
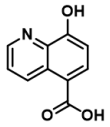

Ion Mode: ESI+
PRO-SIRT2
1080.4135
839.2439
723.2668
738.2366
854.2219
906.3082
ESI+
240000
220000
200000
180000
160000
140000
120000
100000
80000
60000
40000
20000
0
720
740
760
780
800
820
840
860
880
900
920
940
960
980
1000
1020
1040
1060
1080

Table S1. Compounds used in SIRT6 inhibitor screening.

Compound #	Name	Chemical Structure
1	TSA	
2	Nicotinamide (NAM)	
3	EX527	
4	Sirtinol	
5	AGK2	
6	Suramin	
7	SirReal2	
8	Thiomyristoyl	

9	Salermide	
10	Valproic acid	
11	Vorinostat (SAHA)	
12	Sodium butyrate	
13	RG108	
14	Quercetin	
15	3-TYP	
16	NRD167	
17	JQ1	
18	Thioguanine	

19	CPI-455	
20	IOX1	

REFERENCE

1. Curry, A. M.; Cohen, I.; Zheng, S.; Wohlfahrt, J.; White, D. S.; Donu, D.; Cen, Y., Profiling sirtuin activity using Copper-free click chemistry. *Bioorg Chem* **2021,** *117*, 105413.