Sirtuin inhibition and anti-cancer activities of ethyl 2-benzimidazole-5-carboxylate derivatives

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Supporting information

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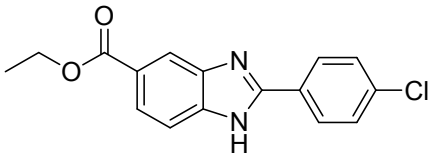
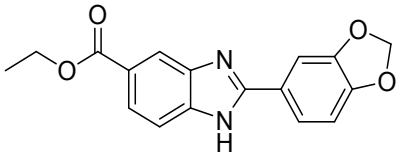
Characterization data for synthesized compounds

Ethyl 2-(4-chlorophenyl)-1H-benzo[d]imidazole-5-carboxylate (BZD9V1)



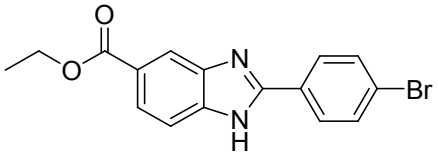
Yield: 85%. ^{1}H NMR (500 MHz; CD_3OD) : 1.43 (3H, t, *J* = 6.9 Hz), 4.38 (2H, q, *J* = 6.9 Hz), 7.56 (2H, d, *J* = 8.4 Hz), 7.63 (1H, d, *J* = 8.4 Hz), 7.94 (2H, d, *J* = 8.4 Hz), 8.05 (1H, dd, *J* = 1.5 Hz, 8.4 Hz), 8.26 (1H, s). ^{13}C NMR (125 MHz, CD_3OD): 14.69, 62.15, 125.43, 126.35, 128.64, 129.20, 129.52, 130.47, 137.94, 154.68, 168.46. ESI-MS: m/z 301.1 (100%); 303.1 (35%) $[M+H]^+$. Anal. Calc. for $C_{16}H_{13}N_2O_2Cl$: C 63.89%; H 4.41%; N 9.32%. Found : C 63.90%; H 4.40%; N 9.30%.

Ethyl 2-(benzo[d][1,3]dioxol-5-yl)-1H-benzo[d]imidazole-5-carboxylate (BZD9Q1)



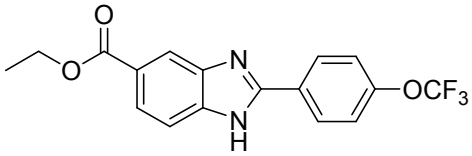
Yield: 85%. ^{1}H NMR (500 MHz; CD_3OD): 1.42 (3H, t, J = 7.2 Hz), 4.38 (2H, t, J = 7.2 Hz), 6.06 (2H, s), 6.99 (1H, d, J = 9 Hz), 7.55 (1H, s), 7.62 (1H, d, J = 9 Hz), 7.93 (1H, d, J = 9 Hz), 8.23 (1H, s). ^{13}C NMR (125 MHz; CD_3OD): 14.70, 62.09, 103.31, 107.98, 108.24, 108.76, 109.79, 121.09, 122.82, 124.40, 125.96, 150.01, 151.50, 168.58. ESI-MS: m/z 312.1 (100%) $[M+H]^+$. Anal. Calc. for $C_{17}H_{14}N_2O_4$: C 65.81%; H 4.60%; N 9.02%. Found : C 65.85%; H 4.64%, N 8.91%.

Ethyl 2-(4-bromophenyl)-1H-benzo[d]imidazole-5-carboxylate (BZD9D1)



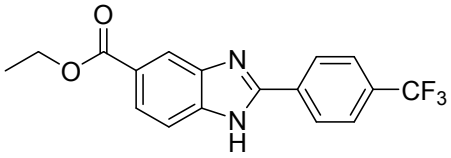
Yield: 94%. ^{1}H NMR (300MHz; DMSO-d_6), δ (ppm): 8.20 (1H, Ar<u>H</u>, s), 8.13 (2H, Ar<u>H</u>, d, *J* = 8.40 Hz), 7.86 (1H, Ar<u>H</u>, dd, *J* = 3.33 Hz, 8.40 Hz), 7.80 (2H, Ar<u>H</u>, d, *J* = 8.40 Hz), 7.68 (1H, Ar<u>H</u>, d, *J* = 8.40 Hz), 4.34 (2H, C<u>H</u>$_2$, q, *J* = 7.10 Hz), 1.35 (3H, C<u>H</u>$_3$, t, *J* = 7.10 Hz). ESI-MS: m/z 344.1 (100%) $[M]^+$; 346.1 (100%) $[M+2]^+$. Anal. Calc. for $C_{16}H_{13}N_2O_2Br$: C 55.67%; H 3.80%; N 8.12%. Found : C 55.30%; H 3.74%, N 8.36%.

Ethyl 2-(4-(trifluoromethoxy)phenyl)-1H-benzo[d]imidazole-5-carboxylate (BZD9H1)



Yield: 77%. ^{1}H NMR (300MHz; DMSO-d_6), δ (ppm): 8.31 (2H, Ar<u>H</u>, d, *J* = 8.40 Hz), 8.21 (1H, Ar<u>H</u>, s), 7.85 (1H, Ar<u>H</u>, dd, *J* = 3.33 Hz; 8.40 Hz), 7.70 (1H, Ar<u>H</u>, d, *J* = 8.40 Hz), 7.60 (2H, Ar<u>H</u>, d, *J* = 8.40 Hz), 4.34 (2H, C<u>H</u>$_2$, q, *J* = 7.10 Hz), 1.36 (3H, C<u>H</u>$_3$, t, *J* = 7.10 Hz). ESI-MS: m/z 351.2 (100%) $[M+H]^+$. Anal. Calc. for $C_{17}H_{13}F_3N_2O_3$: C 58.29%; H 3.74%; N 8.00%. Found : C 58.12%; H 3.62%, N 8.10%.

Ethyl 2-(4-(trifluoromethyl)phenyl)-1H-benzo[d]imidazole-5-carboxylate (BZD9K1)



Yield: 63%. ^{1}H NMR (300MHz; DMSO-d_6), δ (ppm): 8.20 (1H, Ar<u>H</u>, s), 8.13 (2H, Ar<u>H</u>, d, *J* = 8.40 Hz), 7.86 (1H, Ar<u>H</u>, dd, *J* = 3.33 Hz; 8.40 Hz), 7.80 (2H, Ar<u>H</u>, d, *J* = 8.40 Hz), 7.68 (1H, Ar<u>H</u>, d, *J* = 8.40 Hz), 4.34 (2H, C<u>H</u>$_2$, q, *J* = 7.10 Hz), 1.35 (3H, C<u>H</u>$_3$, t, *J* = 7.10 Hz). ESI-MS: m/z 335.2 (100%) $[M+H]^+$. Anal. Calc. for $C_{17}H_{13}F_3N_2O_2$: C 61.08%; H 3.92%; N 8.38%. Found : C 61.34%; H 4.10%, N 8.16%.

Supplementary NMR data

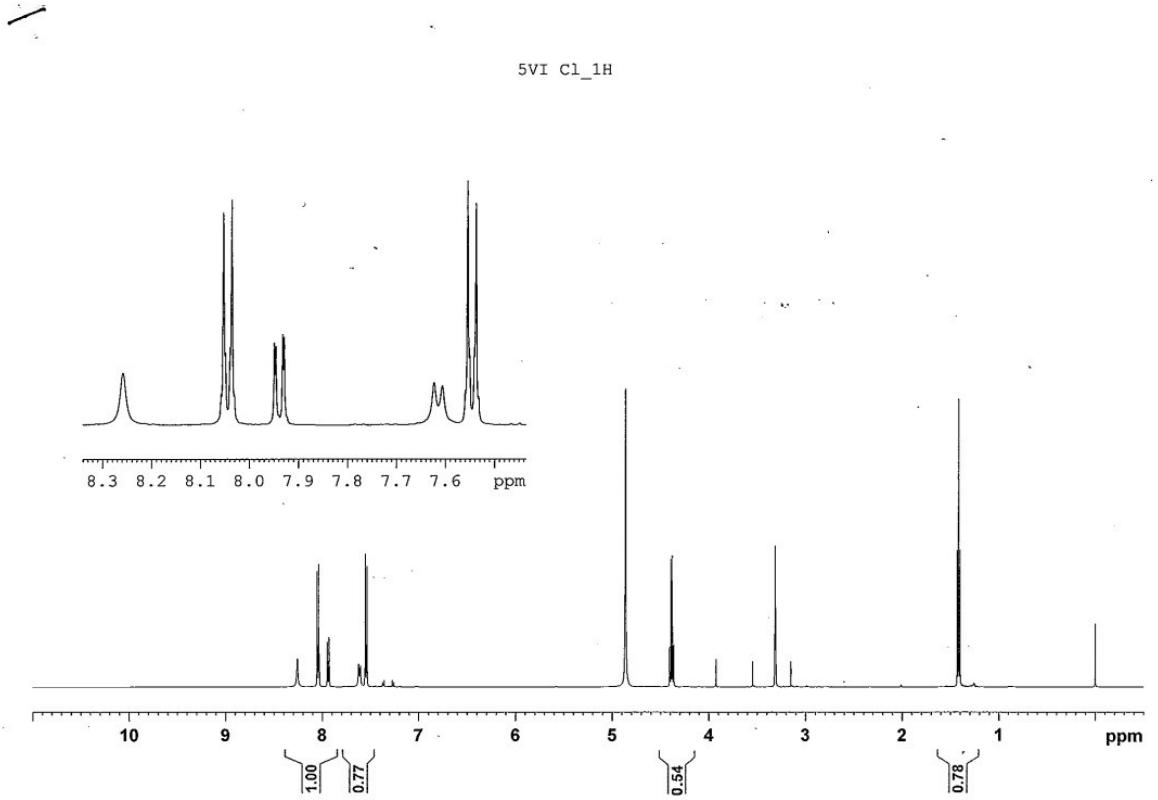


Figure S1. ^{1}H NMR of **BZD9V1**

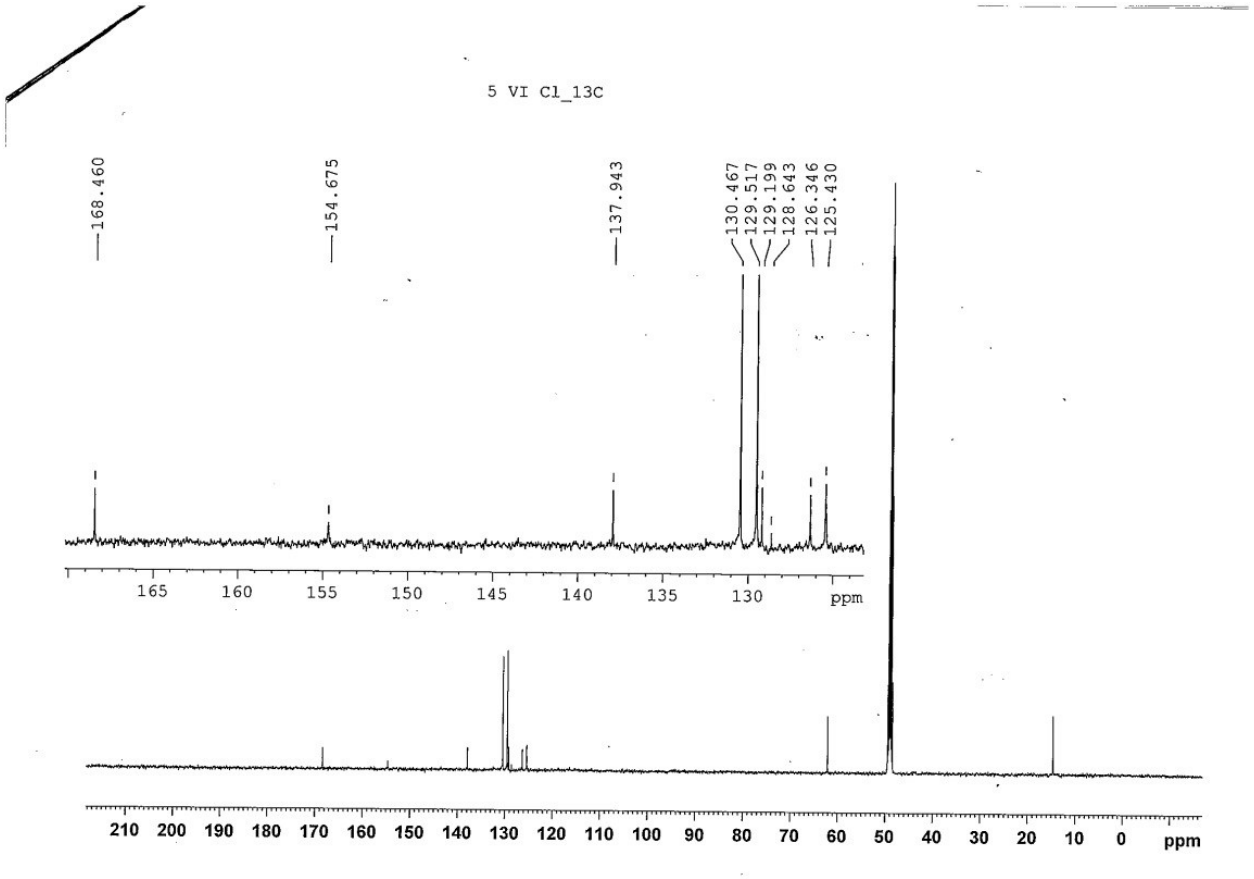


Figure S2. ^{13}C NMR of **BZD9V1**

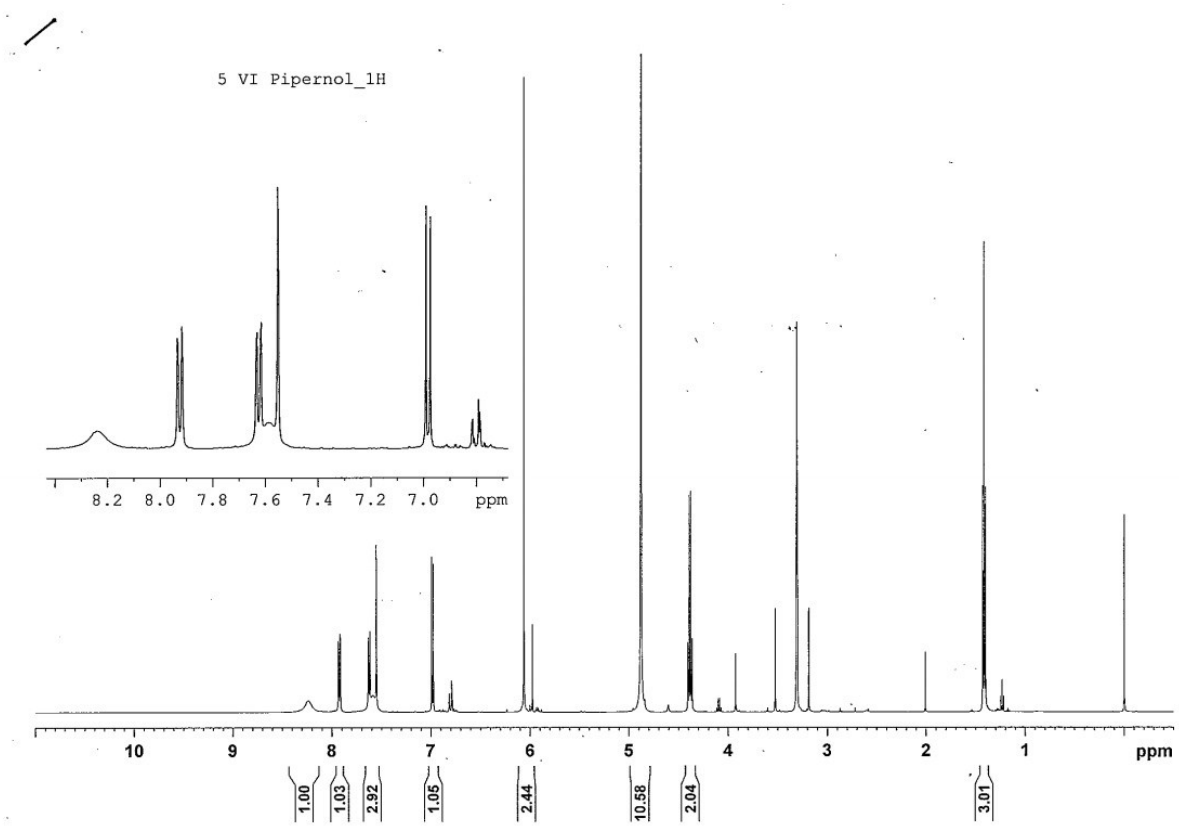


Figure S3. ^{1}H NMR of **BZD9Q1**.

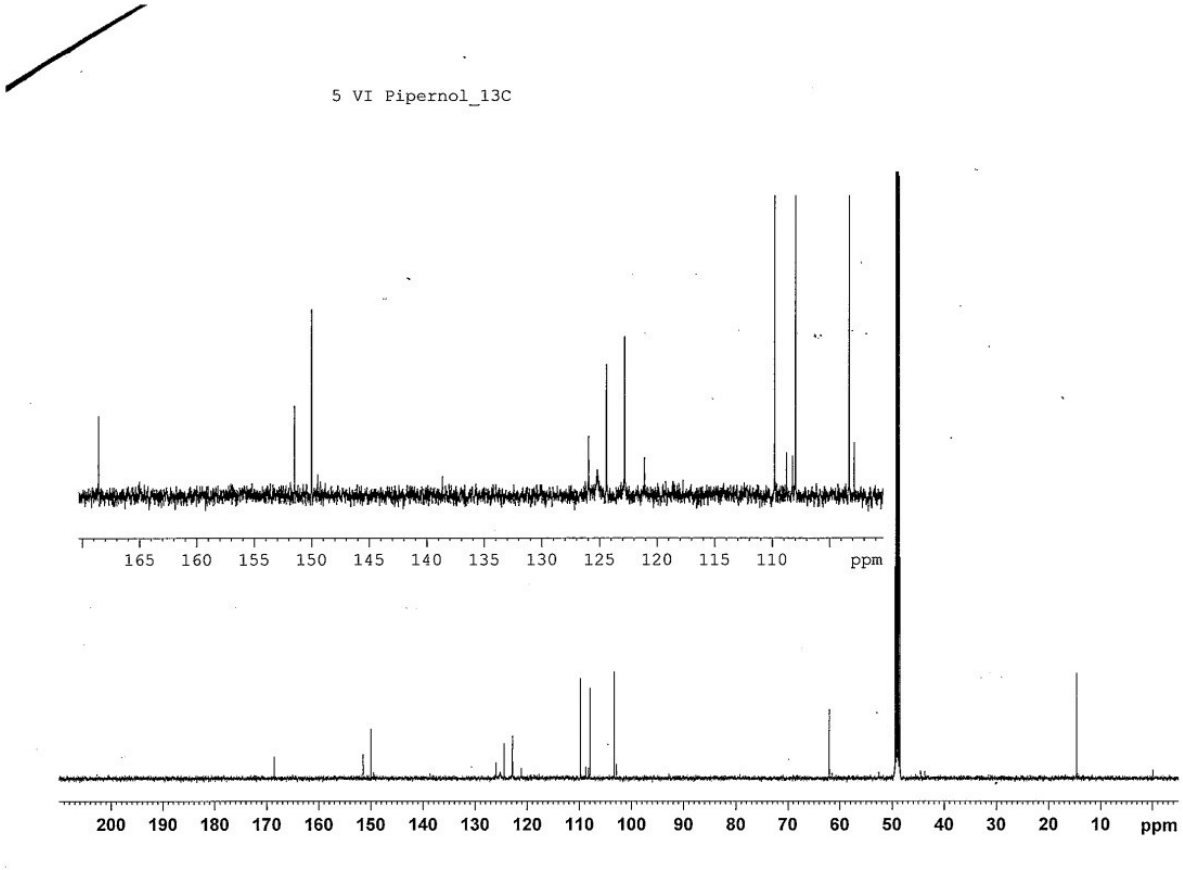


Figure S4. ^{13}C NMR of **BZD9Q1**

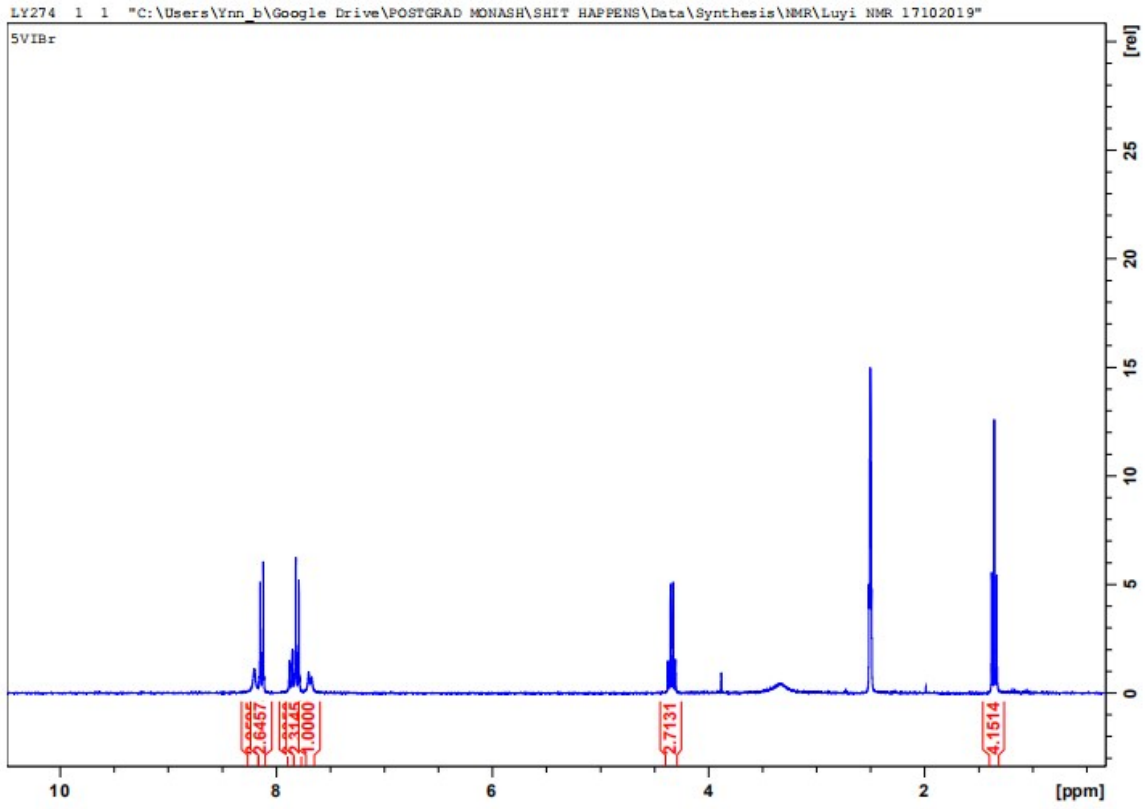


Figure S5. ^{1}H NMR of **BZD9D1**.

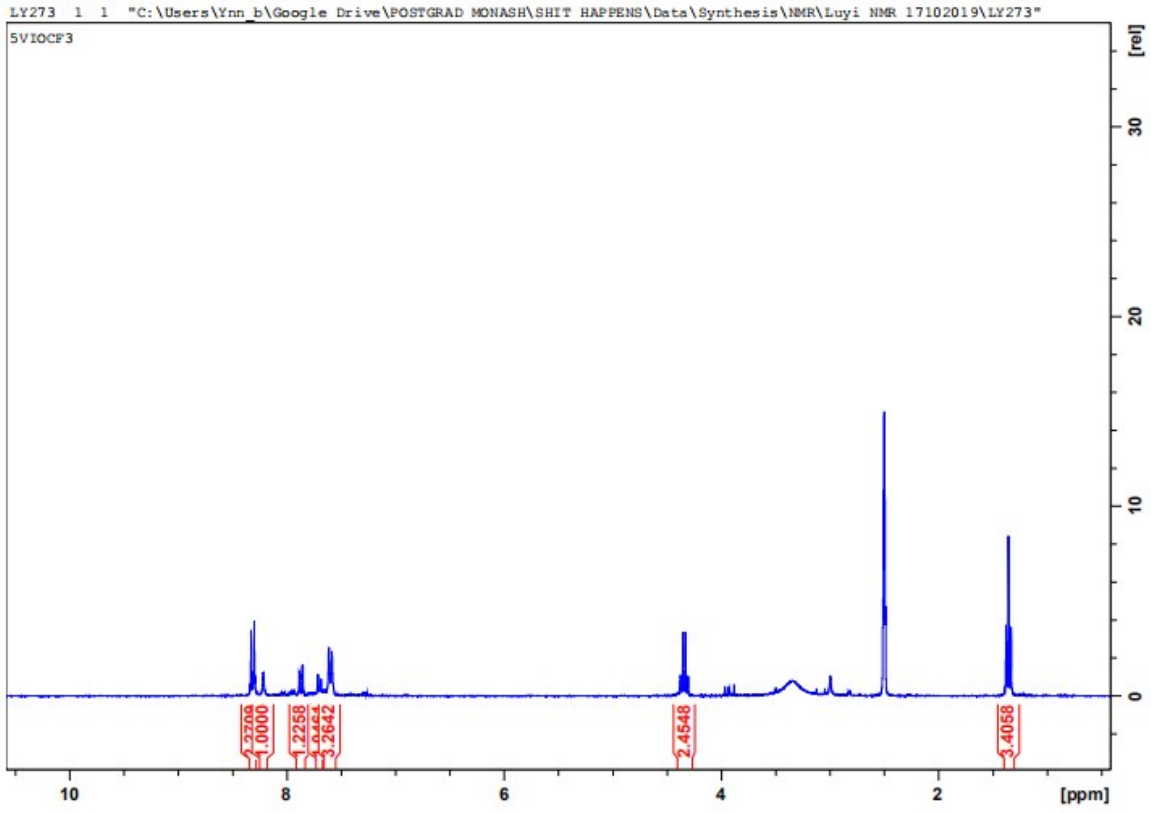


Figure S6. ^{1}H NMR of **BZD9H1**.

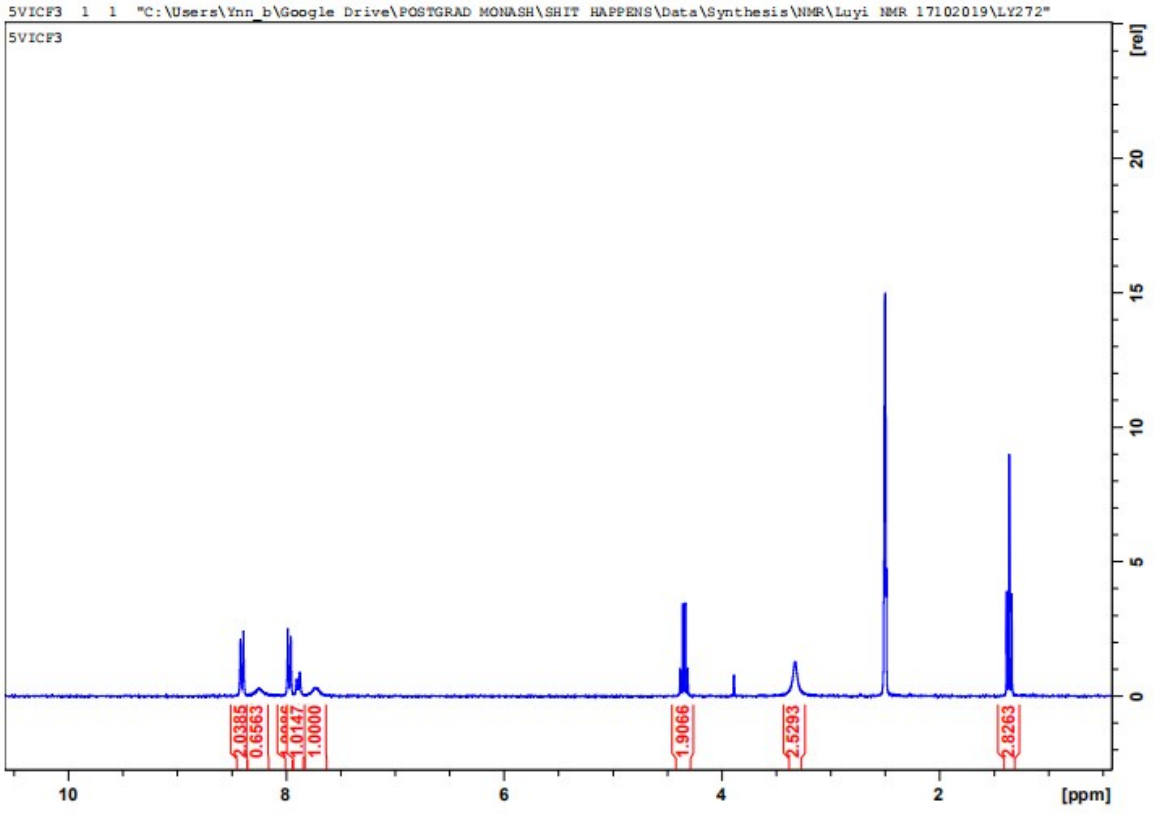


Figure S7. ^{1}H NMR of **BZD9K1**.

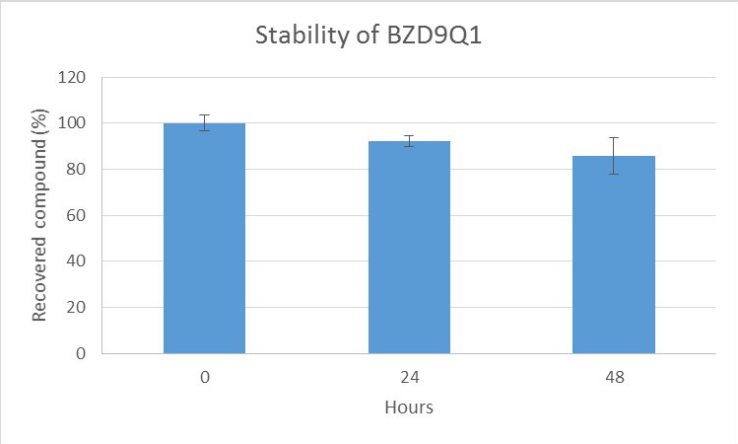


Figure S8. Stability of **BZD9Q1** in DMSO-0.01 M PBS pH 7.4 (5:95). HPLC analysis was performed on Agilent Infinity 1260 using Zorbax SB-C18 (4.6 x 250 mm, 5 micron) column. **BZD9Q1** retention time, t = 4.115 min; captured at 350 nm.

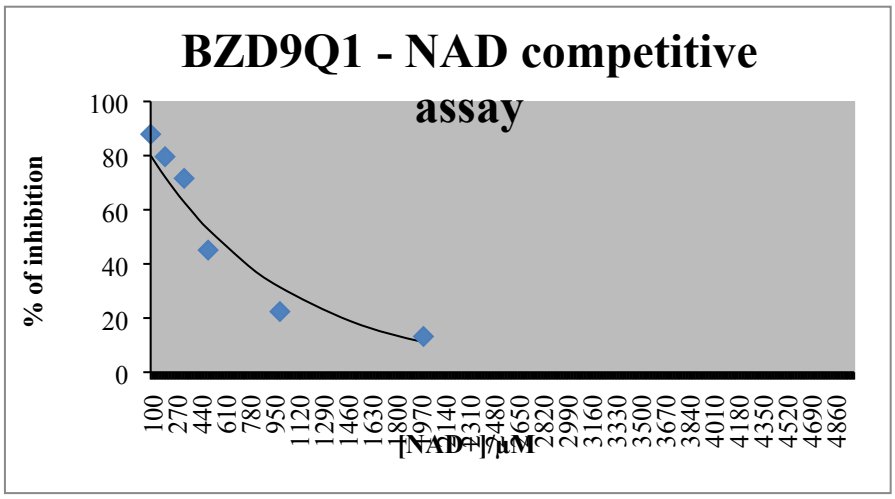


Figure S9. BZD9Q1-NAD competition assay. It was found that SIRT2 inhibition decreased with increasing concentrations of NAD^+ (100, 200, 333, 500, 1000, 2000 µM). The concentration of **BZD9Q1** used in the assay was 10 µM.

Table S1. Cell viability after different time point treatment of **BZD9Q1** on H103 OSCC.

	H103 Treatment		
	24 h GI_{50} (μM)	48 h GI_{50} (μM)	72 h GI_{50} (μM)
BZD9Q1	91	32	5.83
Cisplatin	19	7.21	5.35