Electronic Supplementary Material (ESI) for MedChemComm.

Discovery of a potent and highly fluorescent sirtuin inhibitor

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Supporting information

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Characterization data for synthesized compounds

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

Obtained as beige solid. Yield: 80%; m.p. 177-178 ^{o}C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.44 (3H, t, *J* = 7.1 Hz), 4.44 (2H, q, *J* = 7.1 Hz), 7.50-7.60 (3H, m), 7.64 (1H, s), 7.95 (1H, dd, *J* = 1.5 Hz, 9 Hz), 8.10 (2H, dd, *J* = 9 Hz), 8.30 (1H, s). ^{13}C NMR (125 MHz, $CDCl_3$): 14.70, 62.12, 128.07, 130.28, 130.49, 131.96, 140.27, 143.22, 152.00, 168.54. ESI-MS: m/z 267.2 $[M+H]^+$. Anal. Calc for $C_{16}H_{14}N_2O_2$: C, 72.16%; H, 5.30%; N, 10.52%. Found : C, 72.20%; H, 5.25%; N, 10.50%.

Ethyl 2-p-tolyl-1H-benzo[d]imidazole-5-carboxylate (4b):

Obtained as red-brown solid. Yield: 87%; m.p. 181-182 ^{o}C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.43 (3H, t, *J* = 7.1 Hz), 2.35 (3H, s), 4.41 (2H, q, *J* = 7.1 Hz), 6.95 (2H, d, *J* = 9 Hz), 7.55 (1H, d, *J* = 9 Hz), 7.69 (1H, dd, *J* = 1.5 Hz, 9 Hz), 7.74 (2H, d, *J* = 9 Hz), 8.21 (1H, s). ^{13}C NMR (125 MHz, $CDCl_3$): 14.58, 25.63, 61.59, 115.73, 118.46, 123.99, 125.12, 130.92, 131.62, 149.25, 153.86, 168.90. ESI-MS: m/z 281.1 $[M+H]^+$. Anal. Calc for $C_{17}H_{16}N_2O_2$: C, 72.84%; H, 5.75%; N, 9.99%. Found : C, 72.67%; H, 5.72%; N, 9.90%.

Ethyl 2-(4-tert-butylphenyl)-1H-benzo[d]imidazole-5-carboxylate (4c):

Obtained as brown solid. Yield: 85%; m.p. 200-201 ^{o}C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.31 (9H, s), 1.42 (3H, t, *J* = 7.1 Hz), 4.39 (2H, q, *J* = 7.1 Hz), 6.90 (2H, d, *J* = 9 Hz), 7.55 (1H, d, *J* = 9 Hz), 7.68 (1H, dd, *J* = 1.5 Hz, 9 Hz), 7.74 (2H, d, *J* = 9 Hz), 8.22 (1H, s). ^{13}C NMR (125 MHz, $CDCl_3$): 14.72, 31.90, 34.75, 62.11, 114.73, 119.05, 124.80, 125.33, 129.24, 130.05, 149.22, 153.73, 168.88. ESI-MS: m/z 323.1 $[M+H]^+$. Anal. Calc for $C_{20}H_{22}N_2O_2$: C, 74.51%; H, 6.88%; N, 8.69%. Found : C, 74.37%; H, 6.62%; N, 8.98%.

Ethyl 2-(4-hydroxyphenyl)-1H-benzo[d]imidazole-5-carboxylate (4d):

Obtained as white solid. Yield: 92%; m.p. 209-210 ^{o}C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.45 (3H, t, *J* = 7.2 Hz), 4.43 (2H, q, *J* = 7.2 Hz), 6.88 (1H, d, *J* = 8.4 Hz), 7.49 (2H, d, *J* = 8.4 Hz), 7.59 (2H, d, *J* = 8.4 Hz), 8.08 (1H, dd, *J* = 1.5 Hz, 8.4 Hz), 8.56 (1H, s). ^{13}C NMR (75 MHz, $CDCl_3$): 14.69, 61.56, 105.68, 107.77, 109.20, 111.10, 122.95, 125.60, 153.29, 157.46, 168.58. ESI-MS: m/z 283.1 $[M+H]^+$. Anal. Calc for $C_{16}H_{14}N_2O_3$: C, 68.11%; H, 5.00%; N, 9.91%. Found : C, 68.12%; H, 5.02%; N, 9.88%.

Ethyl 2-(4-methoxyphenyl)-1H-benzo[d]imidazole-5-carboxylate (4e):

Obtained as beige crystal. Yield: 90%; m.p. 188-189 ^{o}C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.43 (3H, t, *J* = 6.9 Hz), 3.88 (3H, s), 4.53 (2H, t, *J* = 6.9 Hz), 7.06 (2H, d, *J* = 9 Hz), 7.47 (1H, d, *J* = 9 Hz), 7.75 (2H, d, *J* = 9 Hz), 8.04 (1H, dd, *J* = 1.5 Hz, 9 Hz), 8.54 (1H, s). ^{13}C NMR (75 MHz, $CDCl_3$): 14.69, 56.22, 61.45, 109.75, 109.98, 121.12, 122.18, 123.40, 150.05, 151.59, 168.67. ESI-MS: m/z 297.1 $[M+H]^+$. Anal. Calc for $C_{17}H_{16}N_2O_3$: C, 68.88%; H, 5.50%; N, 4.47%. Found : C, 68.90%; H, 5.41%; N, 4.50%.

Ethyl 2-(4-(dimethylamino)phenyl)-1H-benzo[d]imidazole-5-carboxylate (4f):

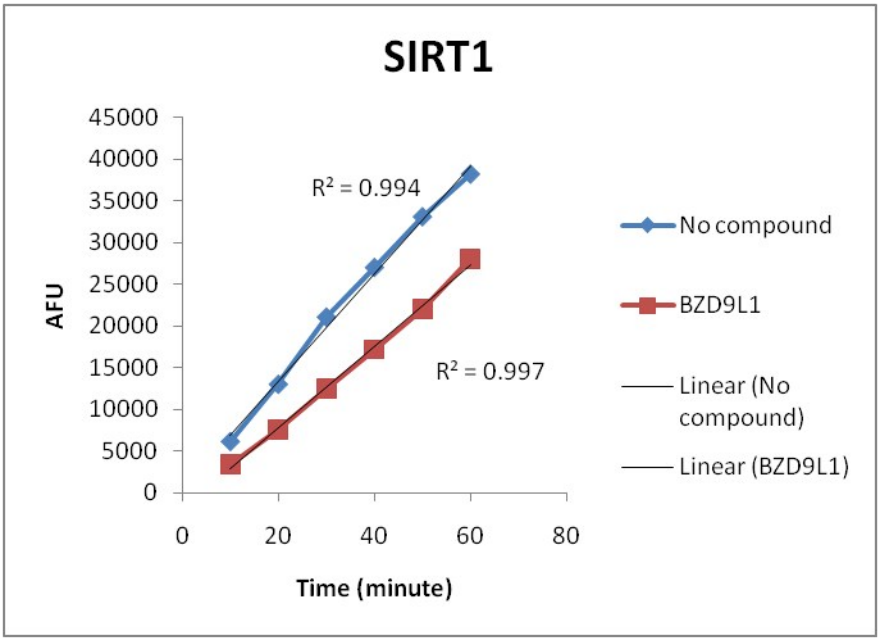
Obtained as brown solid. Yield: 84%; m.p. 220-221 ^{o}C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.42 (3H, t, *J* = 7.1 Hz), 3.05 (3H, s), 4.38 (2H, q, *J* = 7.1 Hz), 6.86 (2H, d, *J* = 9 Hz), 7.55 (1H, d, *J* = 9 Hz), 7.70 (1H, dd, *J* = 1.5 Hz, 9 Hz), 7.73 (2H, d, *J* = 9 Hz), 8.20 (1H, s). ^{13}C NMR (125 MHz, $CDCl_3$): 14.72, 40.30, 62.07, 113.06, 117.27, 124.80, 125.33, 129.24, 129.82, 148.02, 153.69, 168.86. ESI-MS: m/z 310.1 $[M+H]^+$. Anal. Calc for $C_{18}H_{19}N_3O_2$: C, 69.95%; H, 6.17%; N, 13.59%. Found : C, 69.90%; H, 6.21%; N, 13.62%.

Ethyl 2-(4-(piperidin-1-yl)phenyl)-1H-benzo[d]imidazole-5-carboxylate (4g/BZD9L1):

Obtained as brown solid. Yield 83%; m.p. 190-191 ^{o}C. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.38 (3H, t, *J* = 7.0 Hz), 1.67 (6H, t, *J* = 6.5 Hz), 3.16 (4H, t, *J* = 6.5 Hz), 4.43 (2H, q, *J* = 7.0 Hz), 6.72 (2H, d, *J* = 8.5 Hz), 7.54 (1H, d, *J* = 8.5 Hz), 7.88 (1H, dd, *J* = 1.5 Hz, 8.5 Hz), 7.95 (2H, d, *J* = 8.5 Hz), 8.23 (1H, s). ^{13}C NMR : 14.35, 24.19, 25.30, 48.58, 61.02, 114.30, 114.41, 115.91, 124.52, 125.00, 128.48, 135.60, 153.18, 153.89, 166.96. ESI-MS: m/z 350.2 $[M+H]^+$. Anal. Calc. for $C_{21}H_{23}N_3O_2$: C, 72.18%; H, 6.63%; N, 12.03%. Found : C, 72.15; H, 6.61; N, 12.04%.

Ethyl 2-(4-morpholinophenyl)-1H-benzo[d]imidazole-5-carboxylate (4h):

Obtained as orange-brown solid. Yield 73%; m.p. 204-205 ^{o}C. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.44 (3H, t, *J* = 7.0 Hz), 3.22 (4H, t, *J* = 5.0), 3.85 (4H, t, *J* = 5.0 Hz), 4.43 (2H, q, *J* = 7.0 Hz), 6.80 (2H, d, *J* = 8.5 Hz), 7.47 (1H, d, *J* = 8.5 Hz), 7.90 (1H, dd, *J* = 1.5 Hz, 8.5 Hz), 7.98 (1H, d, 2H, d, *J* = 8.5 Hz), 8.27 (1H, s). ^{13}C NMR : 14.34, 47.97, 60.90, 66.67, 112.08, 114.54, 116.01, 124.70, 123.65, 127.48, 135.76, 151.22, 153.88, 167.95. ESI-MS: m/z 352.2 $[M+H]^+$. Anal. Calc. for $C_{20}H_{21}N_3O_3$: C, 68.36%; H, 6.02%; N, 11.96%. Found : C, 68.31; H, 6.07; N, 11.93%.



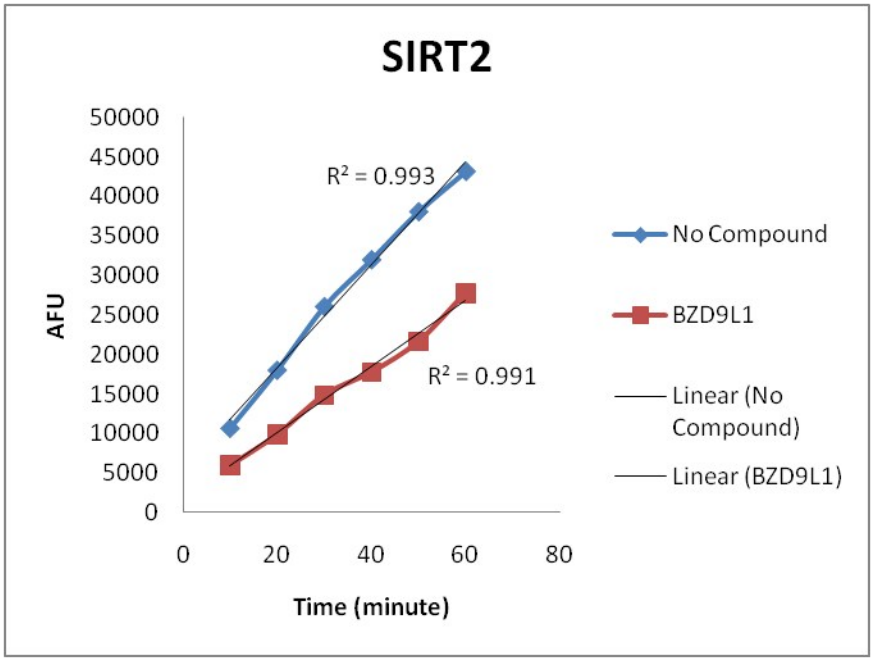


Fig. S1. Plots of product formation versus time in the absence and presence of 10 μM of **BZD9L1**.

AFU stands for arbitrary fluorescence unit.

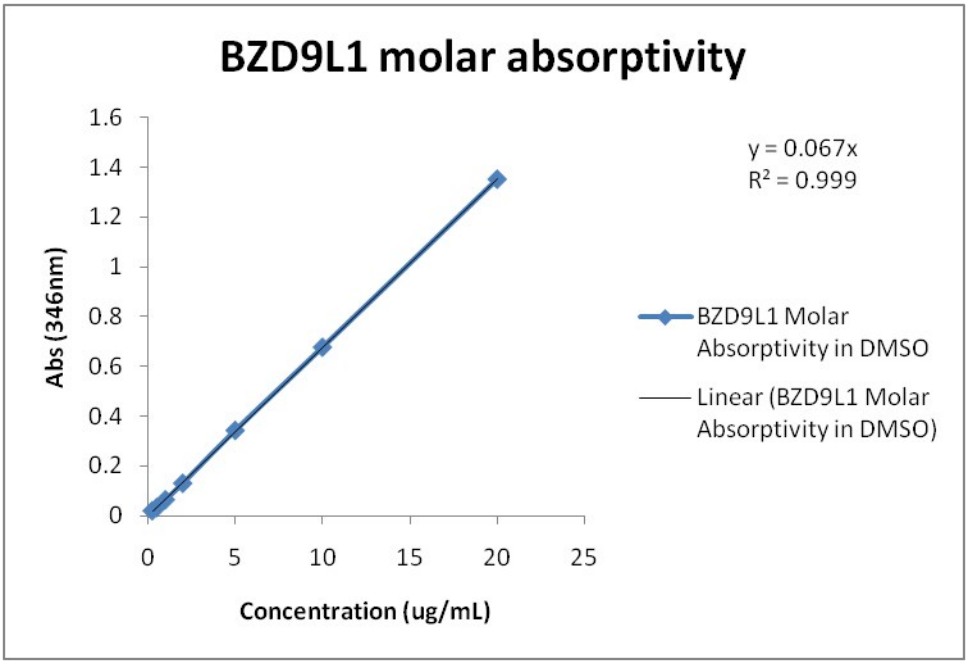


Fig. S2. Plot of absorbance (346 nm) versus concentration of **BZD9L1** in DMSO. Molar absorptivity (molar extinction coefficient, ε) was determined by the gradient of the slope using Beer Lambert equation. Absorbance readings were taken in a quartz cuvette (10 mm path length), using Agilent 8453 UV-visible spectrophotometer. The excitation wavelength was set at 346 nm.

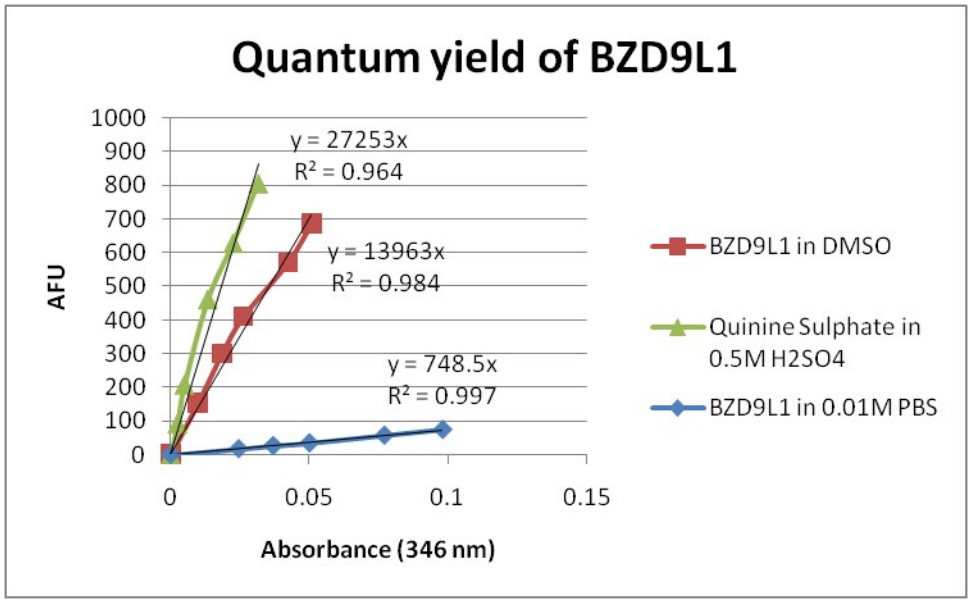


Fig. S3. Quantum yield (Φ) determination of **BZD9L1**. Quinine sulphate was used as reference. AFU stands for arbitrary fluorescence unit.

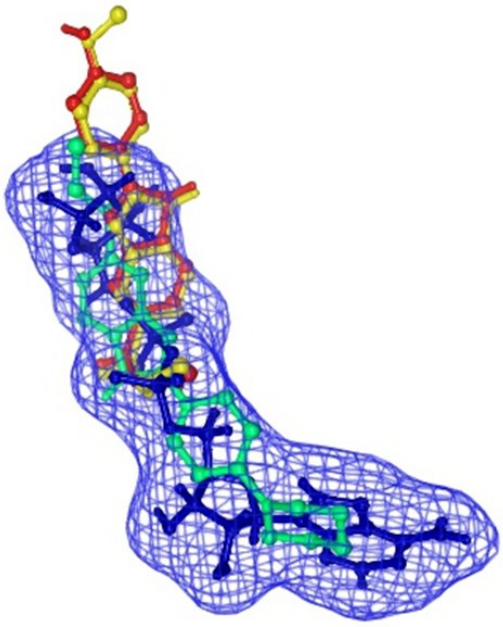
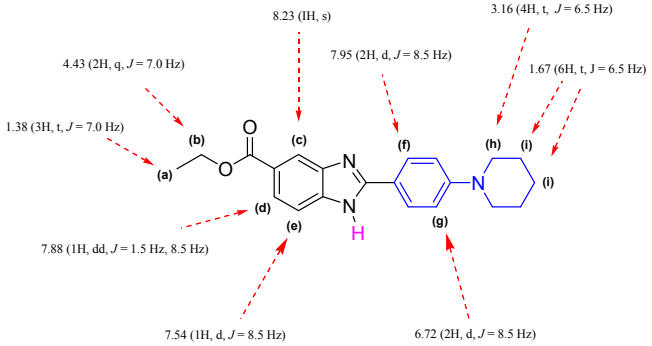


Fig. S4. BZD9L1 (green), **4d** (red) and **4e** (yellow) were docked into the active site of SIRT2 (PDB code: 3ZGV). It showed that **BZD9L1** were optimally fitted into the ADPr binding site (blue) while the phenolic substituent (**4d**) and the anisole substituent (**4e**) were shifted out from the ADPr binding site. This resulted in less favourable complexes and weaker inhibitory SIRT2 activities for **4d** and **4e**.

	CCRF-CEM		HCT116		MDA-MB-468	
	Cell Viability (%)	S.D. (%)	Cell Viability (%)	S.D. (%)	Cell Viability (%)	S.D. (%)
DMSO	100.00	5.54	100.00	6.01	100.00	8.06
4a	58.29	2.24	29.69	9.44	49.27	6.83
4b	61.24	5.44	48.40	12.80	89.08	0.75
4c	82.16	8.59	54.19	4.36	54.62	8.32
4d	85.49	5.37	77.07	3.59	76.48	12.39
4e	87.37	4.66	75.60	2.10	82.76	2.24
4f	49.45	3.41	29.74	1.41	31.10	0.79
4g/BZD9L1	36.26	0.34	15.90	2.04	33.98	0.49
4h	37.81	11.33	54.48	3.93	42.58	4.89

Table S1. Cell viability after 72h treatment of synthesized compounds **4a-h** against HCT-116, MDA-MB-468 and CCRF-CEM cells.

Supplementary NMR data



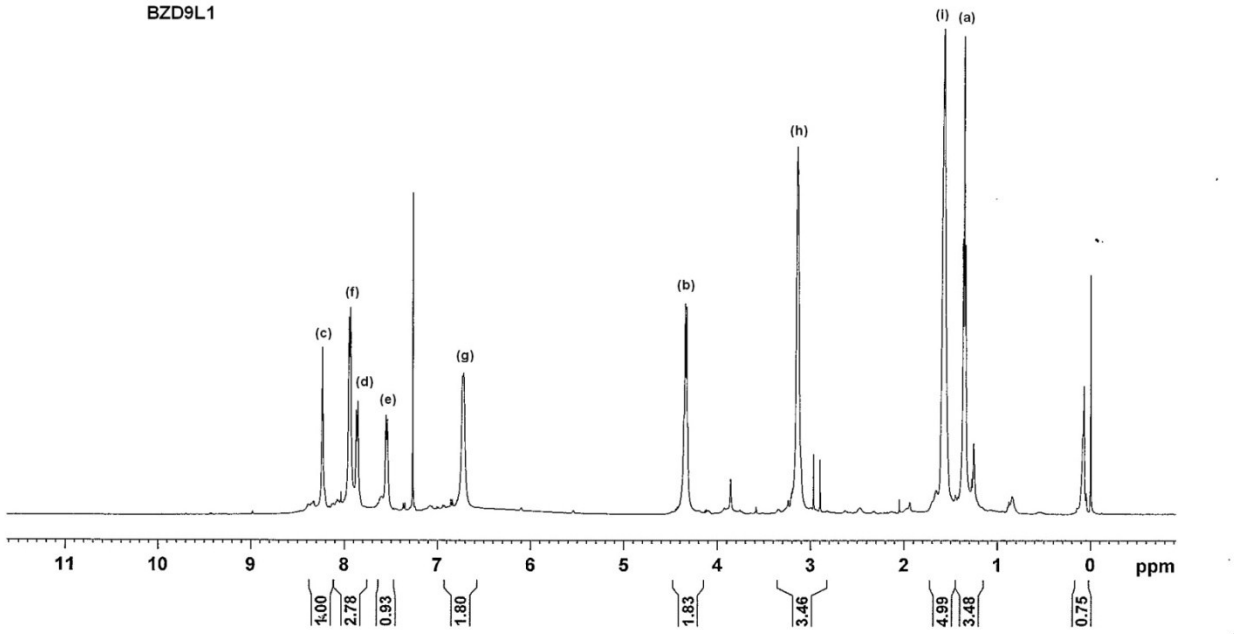


Fig. S5. ^{1}H NMR of **BZD9L1**

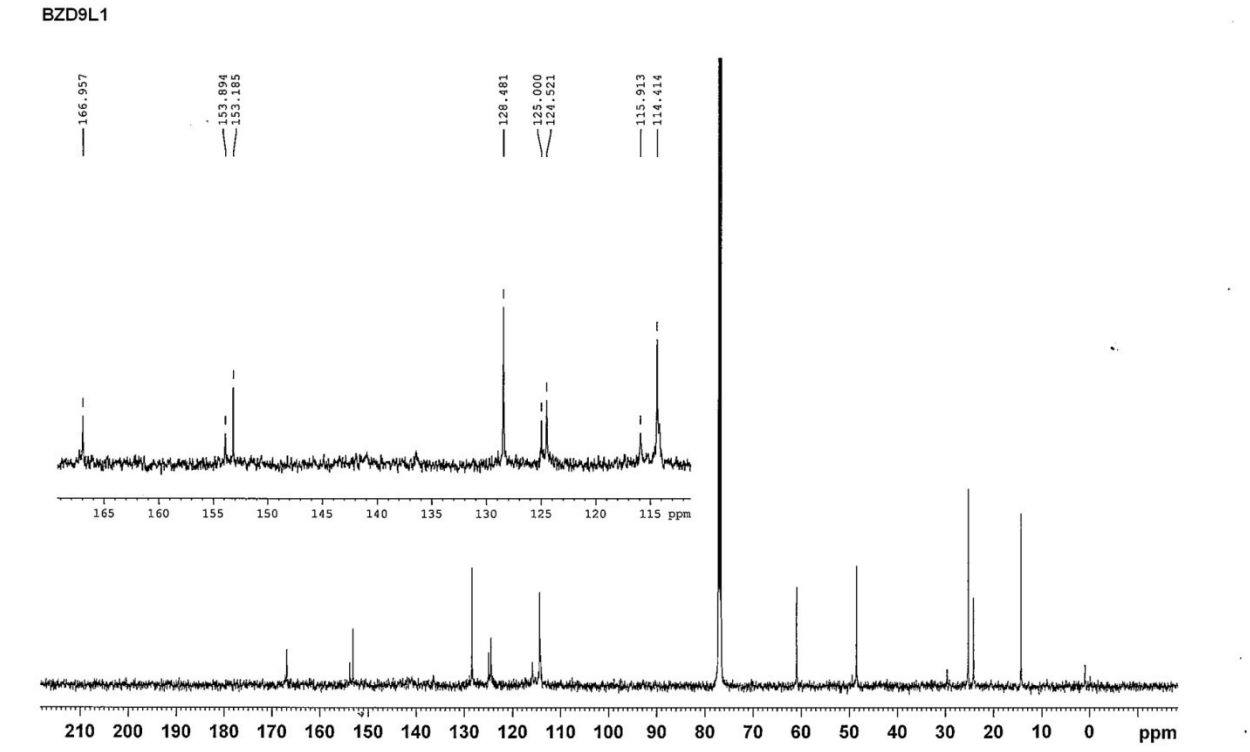


Fig. S6. ^{13}C NMR of **BZD9L1**

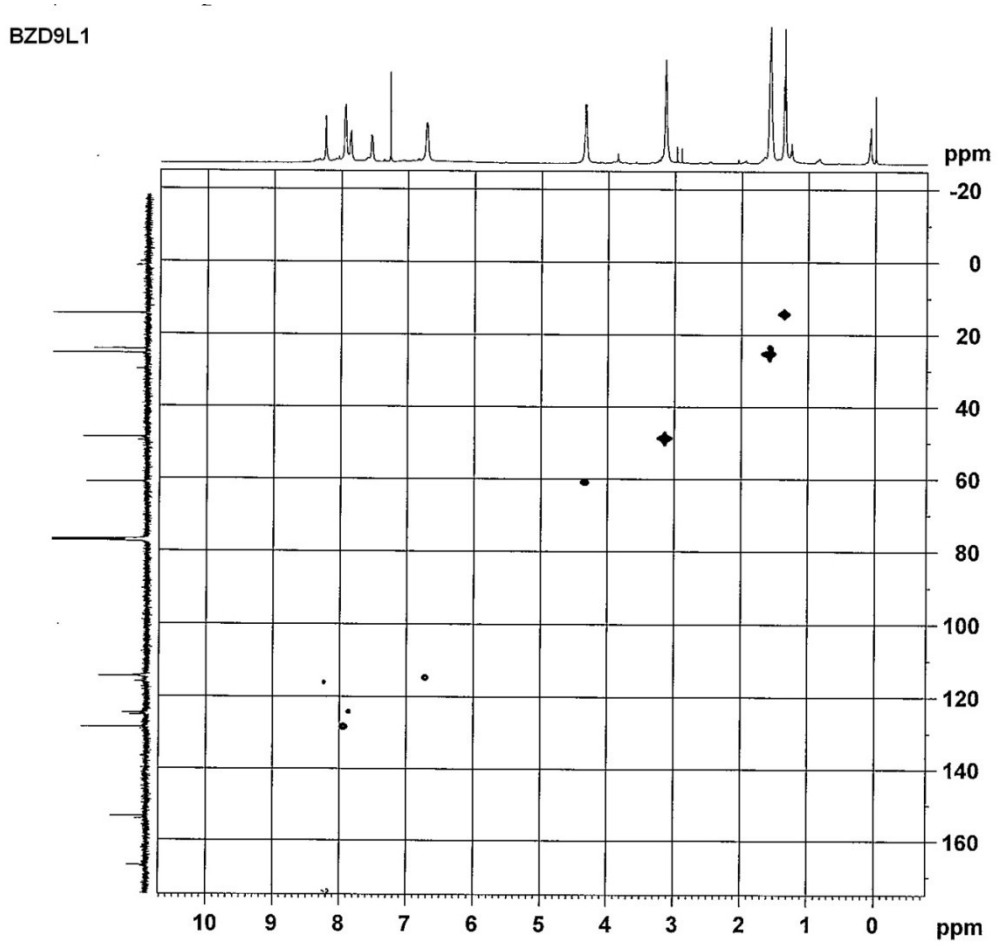


Fig. S7. 2D HMQC for **BZD9L1**.

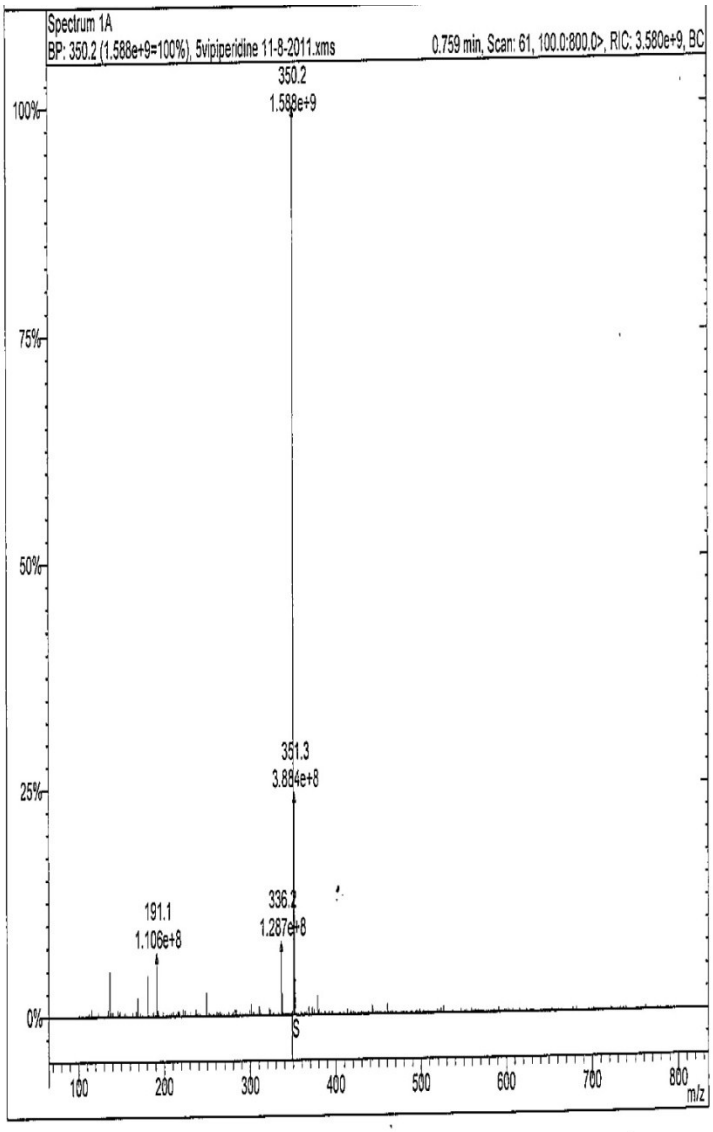


Fig. S8. Direct infusion MS data for **BZD9L1**.