

## Potent sirtuin inhibition with 1,2,5-trisubstituted benzimidazoles

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10. **Figure S9.** SIRT1, SIRT2 and SIRT3 inhibitory plots in the presence of different concentrations of **5i**. Half maximal inhibitory concentration (IC<sub>50</sub>) values were determined from these curves. Error bars showed S.D.

## Characterization data

### *Ethyl 1-(2-(4-(4-(ethoxycarbonyl)-2-aminophenyl)piperazin-1-yl)ethyl)-2-(4-hydroxy-3-methoxyphenyl)-1H-benzo[d]imidazole-5-carboxylate (5h)*

Yield: 65%; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 1.36 (3H, t, *J* = 7.2 Hz), 1.42 (3H, t, *J* = 7.2 Hz), 2.60-3.00 (10H, m), 3.70 (3H, s), 4.33 (2H, q, *J* = 7.2 Hz), 4.30-4.50 (2H, m), 4.42 (2H, q, *J* = 7.2 Hz), 6.67 (1H, d, *J* = 9.0 Hz), 6.91 (1H, dd, *J* = 1.5 Hz, 9.0 Hz), 6.95 (1H, d, *J* = 9.0 Hz), 7.40 (1H, s), 7.45 (1H, dd, *J* = 1.5 Hz, 9.0 Hz), 7.50 (1H, s), 8.04 (1H, dd, *J* = 1.5 Hz, 9.0 Hz), 8.13 (1H, d, *J* = 9.0 Hz), 8.52 (1H, s). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>): 14.35, 14.38, 42.86, 50.31, 53.85, 56.11, 57.25, 60.70, 60.97, 108.89, 109.18, 114.45, 116.06, 116.35, 118.17, 118.96, 120.59, 122.72, 124.52, 124.90, 126.29, 127.45, 128.90, 133.81, 140.76, 143.05, 145.09, 151.79, 167.03, 167.09. ESI-MS: *m/z* 588.2 [M+H]<sup>+</sup>. Anal. Calc for C<sub>32</sub>H<sub>37</sub>N<sub>5</sub>O<sub>6</sub>: C, 65.40%; H, 6.35%; N, 11.92%. Found: C, 65.63%; H, 6.22%; N, 11.90%.

### *Ethyl 1-(2-(4-(2-amino-4-(ethoxycarbonyl)phenyl)piperazin-1-yl)ethyl)-2-(4-(dimethylamino)phenyl)-1H-benzo[d]imidazole-5-carboxylate (5i)*

Yield: 74%; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 1.36 (3H, t, *J* = 7.2 Hz), 1.43 (3H, t, *J* = 7.2 Hz), 2.50-3.00 (10H, m), 3.06 (6H, s), 4.32 (2H, q, *J* = 7.2 Hz), 4.30-4.50 (2H, m), 4.41 (2H, q, *J* = 7.2 Hz), 6.70 (1H, d, *J* = 9.0 Hz), 6.81 (2H, d, *J* = 9.0 Hz), 6.94 (1H, d, *J* = 9.0 Hz), 7.39 (1H, s), 7.44 (1H, dd, *J* = 1.5 Hz, 9.0 Hz), 7.70 (2H, d, *J* = 9.0 Hz), 8.02 (1H, dd, *J* = 1.5 Hz, 9.0 Hz), 8.52 (1H, s). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>): 14.38, 14.40, 29.70, 40.22, 50.15, 53.97, 57.07, 60.70, 60.81, 109.20, 111.81, 115.34, 116.08, 116.75, 118.99, 120.26, 121.52, 124.73, 128.89, 129.21, 130.42, 139.70, 140.89, 143.01, 153.85, 167.20, 167.38. ESI-MS: *m/z* 585.2 [M+H]<sup>+</sup>. Anal. Calc for C<sub>33</sub>H<sub>40</sub>N<sub>6</sub>O<sub>4</sub>: C, 67.79%; H, 6.90%; N, 14.37%. Found: C, 67.68%; H, 7.00%; N, 14.41%.

***Ethyl 1-(2-(4-(2-amino-4-(ethoxycarbonyl)phenyl)piperazin-1-yl)ethyl)-2-(4-(piperidin-1-yl)phenyl)-1H-benzof[d]imidazole-5-carboxylate (5j)***

Yield: 72%; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 1.36 (3H, t, *J* = 7.2 Hz), 1.42 (3H, t, *J* = 7.2 Hz), 1.60-1.80 (6H, m), 2.50-2.90 (10H, m), 3.30 (4H, t, *J* = 7.0 Hz), 4.32 (2H, q, *J* = 7.2 Hz), 4.30-4.50 (2H, m), 4.41 (2H, q, *J* = 7.2 Hz), 6.88 (1H, d, *J* = 9.0 Hz), 6.93 (1H, d, *J* = 9.0 Hz), 7.00 (2H, d, *J* = 9.0 Hz), 7.39 (1H, s), 7.43 (1H, dd, *J* = 1.5 Hz, 9.0 Hz), 7.70 (2H, d, *J* = 9.0 Hz), 8.01 (1H, dd, *J* = 1.5 Hz, 9.0 Hz), 8.51 (1H, s). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>): 14.36, 14.39, 18.41, 24.28, 31.42, 42.84, 50.66, 53.92, 57.02, 60.64, 60.81, 109.45, 113.24, 115.13, 115.97, 116.59, 118.83, 120.46, 121.73, 123.87, 128.18, 129.33, 130.34, 139.00, 140.79, 142.77, 152.80, 167.22, 167.35. ESI-MS: *m/z* 625.2 [M+H]<sup>+</sup>. Anal. Calc for C<sub>36</sub>H<sub>44</sub>N<sub>6</sub>O<sub>4</sub>: C, 69.21%; H, 7.10%; N, 13.45%. Found: C, 69.15%; H, 7.05%; N, 13.60%.

***Ethyl 1-octyl-2-phenyl-1H-benzof[d]imidazole-5-carboxylate (8a)***

Yield: 88%; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 0.89 (3H, t, *J* = 8.0 Hz), 1.30-1.60 (15, m), 3.77 (2H, t, *J* = 5.5 Hz), 4.42 (2H, q, *J* = 7.2 Hz), 6.70-7.60 (6H, m), 7.82 (1H, dd, *J* = 1.5 Hz, 9.0 Hz), 8.14 (1H, s). ESI-MS: *m/z* 379.3 [M+H]<sup>+</sup>. Anal. Calc for C<sub>24</sub>H<sub>30</sub>N<sub>2</sub>O<sub>2</sub>: C, 76.16%; H, 7.99%; N, 7.40%. Found: C, 76.05%; H, 7.95%; N, 7.51%.

***Ethyl 2-(4-chlorophenyl)-1-octyl-1H-benzof[d]imidazole-5-carboxylate (8b)***

Yield: 90%; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 0.88 (3H, t, *J* = 8.0 Hz), 1.30-1.60 (15, m), 3.86 (2H, t, *J* = 5.5 Hz), 4.43 (2H, q, *J* = 7.2 Hz), 6.86 (2H, d, *J* = 9.0 Hz), 7.34 (1H, d, *J* = 9.0 Hz), 7.61 (2H, d, *J* = 9.0 Hz), 7.75 (1H, dd, *J* = 1.5 Hz, 9.0 Hz), 8.45 (1H, s). ESI-MS: *m/z* 413.2 [M+H]<sup>+</sup>. Anal. Calc for C<sub>24</sub>H<sub>29</sub>N<sub>2</sub>O<sub>2</sub>Cl: C, 69.80%; H, 7.08%; N, 6.78%. Found: C, 69.67%; H, 7.04%; N, 6.91%.

***Ethyl 2-(2,4-dihydroxyphenyl)-1-octyl-1H-benzo[d]imidazole-5-carboxylate (8c)***

Yield: 75%; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 0.88 (3H, t, *J* = 8.0 Hz), 1.30-1.60 (15, m), 3.88 (2H, t, *J* = 5.5 Hz), 4.44 (2H, q, *J* = 7.2 Hz), 6.69 (1H, s), 6.90 (1H, dd, *J* = 1.5 Hz, 9.0 Hz), 7.44 (1H, d, *J* = 9.0 Hz), 7.52 (1H, d, *J* = 9.0 Hz), 8.18 (1H, dd, *J* = 1.5 Hz, 9.0 Hz), 8.54 (1H, s). ESI-MS: *m/z* 411.3 [M+H]<sup>+</sup>. Anal. Calc for C<sub>24</sub>H<sub>30</sub>N<sub>2</sub>O<sub>4</sub>: C, 70.22%; H, 7.37%; N, 6.82%. Found: C, 70.31%; H, 7.34%; N, 6.90%.

***Ethyl 1-octyl-2-(4-(trifluoromethoxy)phenyl)-1H-benzo[d]imidazole-5-carboxylate (8d)***

Yield: 84%; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 0.88 (3H, t, *J* = 8.0 Hz), 1.30-1.60 (15, m), 3.85 (2H, t, *J* = 5.5 Hz), 4.43 (2H, q, *J* = 7.2 Hz), 7.55 (1H, d, *J* = 9.0 Hz), 7.80 (2H, d, *J* = 9.0 Hz), 7.98 (2H, d, *J* = 9.0 Hz), 8.10 (1H, dd, *J* = 1.5 Hz, 9.0 Hz), 8.56 (1H, s). ESI-MS: *m/z* 463.2 [M+H]<sup>+</sup>. Anal. Calc for C<sub>25</sub>H<sub>29</sub>N<sub>2</sub>O<sub>3</sub>F<sub>3</sub>: C, 64.92%; H, 6.32%; N, 6.06%. Found: C, 64.94%; H, 6.33%; N, 6.10%.

***Ethyl 1-octyl-2-p-tolyl-1H-benzo[d]imidazole-5-carboxylate (8e)***

Yield: 82%; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 0.90 (3H, t, *J* = 8.0 Hz), 1.30-1.60 (15, m), 2.35 (3H, s), 3.87 (2H, t, *J* = 5.5 Hz), 4.43 (2H, q, *J* = 7.2 Hz), 6.93 (2H, d, *J* = 9.0 Hz), 7.49 (1H, d, *J* = 9.0 Hz), 7.63 (1H, dd, *J* = 1.5 Hz, 9.0 Hz), 7.73 (2H, d, *J* = 9.0 Hz), 8.49 (1H, s). ESI-MS: *m/z* 393.3 [M+H]<sup>+</sup>. Anal. Calc for C<sub>25</sub>H<sub>32</sub>N<sub>2</sub>O<sub>2</sub>: C, 76.49%; H, 8.22%; N, 7.14%. Found: C, 76.56%; H, 8.30%; N, 7.29%.

***Ethyl 2-(benzo[d][1,3]dioxol-5-yl)-1-octyl-1H-benzo[d]imidazole-5-carboxylate (8f)***

Yield: 91%; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 0.89 (3H, t, *J* = 8.0 Hz), 1.30-1.60 (15, m), 3.85 (2H, t, *J* = 5.5 Hz), 4.44 (2H, q, *J* = 7.2 Hz), 6.10 (2H, s), 7.33 (1H, d, *J* = 9.0 Hz), 7.40-7.55

(3H, m), 8.08 (1H, d,  $J = 9.0$  Hz), 8.53 (1H, s). ESI-MS:  $m/z$  423.3  $[M+H]^+$ . Anal. Calc for  $C_{25}H_{30}N_2O_4$ : C, 71.07%; H, 7.16%; N, 6.63%. Found: C, 71.12%; H, 8.20%; N, 6.61%.

***Ethyl 2-(4-(5-(4-fluorophenyl)pyridin-3-yl)phenyl)-1-octyl-1H-benzo[d]imidazole-5-carboxylate (8g)***

Yield: 70%;  $^1H$  NMR (500 MHz,  $CDCl_3$ ):  $\delta$  0.88 (3H, t,  $J = 8.0$  Hz), 1.30-1.60 (15, m), 3.85 (2H, t,  $J = 5.5$  Hz), 4.44 (2H, q,  $J = 7.2$  Hz), 7.03 (2H, d,  $J = 9.5$  Hz), 7.40-7.50 (3H, m), 8.10-8.20 (2H, m), 8.57 (1H, s), 8.76 (2H, s). ESI-MS:  $m/z$  550.2  $[M+H]^+$ . Anal. Calc for  $C_{35}H_{36}N_3O_2F$ : C, 76.48%; H, 6.60%; N, 7.64%. Found: C, 76.75%; H, 6.81%; N, 7.44%.

***Ethyl 2-(4-hydroxy-3-methoxyphenyl)-1-octyl-1H-benzo[d]imidazole-5-carboxylate (8h)***

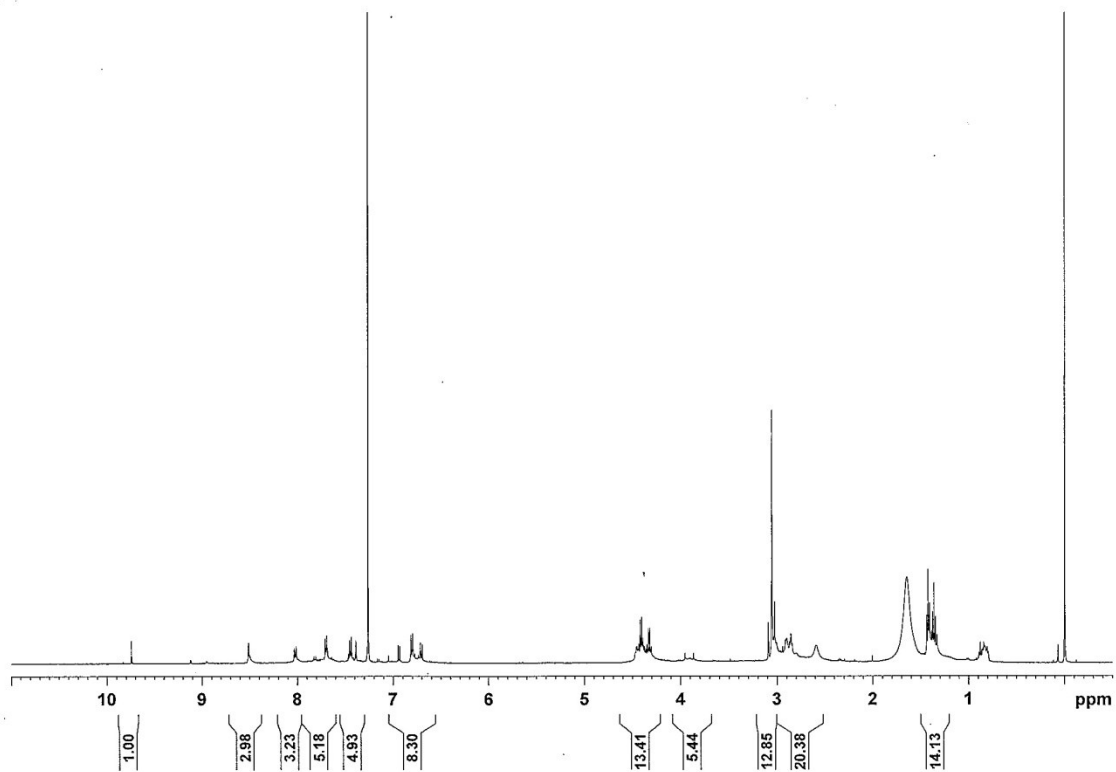
Yield: 89%;  $^1H$  NMR (500 MHz,  $CDCl_3$ ):  $\delta$  0.88 (3H, t,  $J = 8.0$  Hz), 1.30-1.60 (15, m), 3.87 (2H, t,  $J = 5.5$  Hz), 4.43 (2H, q,  $J = 7.2$  Hz), 7.04 (1H, d,  $J = 9.0$  Hz), 7.23 (1H, dd,  $J = 1.5$  Hz, 9.0 Hz), 7.37 (1H, s), 7.44 (1H, d,  $J = 8.1$  Hz), 8.05 (1H, dd,  $J = 1.5$  Hz, 9.0 Hz), 8.55 (1H, s). ESI-MS:  $m/z$  425.3  $[M+H]^+$ . Anal. Calc for  $C_{25}H_{32}N_2O_4$ : C, 70.73%; H, 7.60%; N, 6.60%. Found: C, 70.70%; H, 7.61%; N, 6.64%.

***Ethyl 2-(4-(dimethylamino)phenyl)-1-octyl-1H-benzo[d]imidazole-5-carboxylate (8i)***

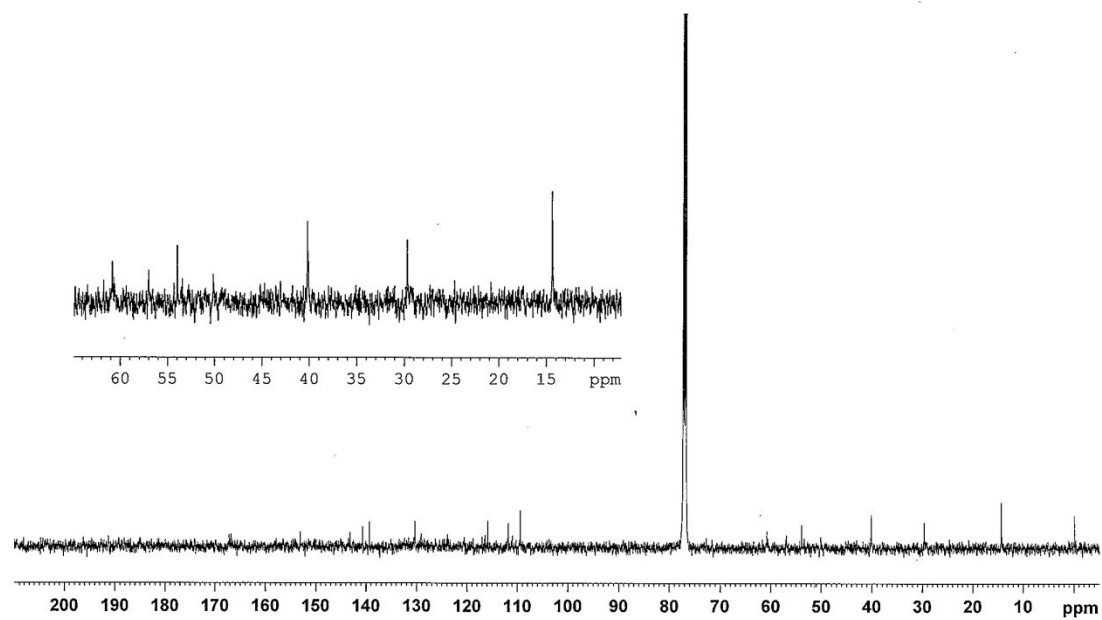
Yield: 80%;  $^1H$  NMR (500 MHz,  $CDCl_3$ ):  $\delta$  0.88 (3H, t,  $J = 8.0$  Hz), 1.30-1.60 (15, m), 3.07 (3H, s), 3.85 (2H, t,  $J = 5.5$  Hz), 4.42 (2H, q,  $J = 7.2$  Hz), 6.89 (2H, d,  $J = 9$  Hz), 7.55 (1H, d,  $J = 9$  Hz), 7.69 (1H, dd,  $J = 1.5$  Hz, 9.0 Hz), 7.75 (2H, d,  $J = 9.0$  Hz), 8.25 (1H, s). ESI-MS:  $m/z$  422.3  $[M+H]^+$ . Anal. Calc for  $C_{26}H_{35}N_3O_2$ : C, 74.07%; H, 8.37%; N, 9.97%. Found: C, 74.05%; H, 7.36%; N, 10.00%.

***Ethyl 1-octyl-2-(4-(piperidin-1-yl)phenyl)-1H-benzo[d]imidazole-5-carboxylate (8j)***

Yield: 83%; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 0.89 (3H, t, *J* = 8.0 Hz), 1.30-1.60 (15, m), 1.70 (6H, t, *J* = 6.0 Hz), 3.20 (4H, t, *J* = 6.0 Hz), 3.86 (2H, t, *J* = 5.5 Hz), 4.42 (2H, q, *J* = 7.2 Hz), 6.69 (2H, d, *J* = 9.0 Hz), 7.51 (1H, d, *J* = 9.0 Hz), 7.88 (1H, dd, *J* = 1.5 Hz, 9.0 Hz), 7.95 (2H, d, *J* = 9.0 Hz), 8.26 (1H, s). ESI-MS: *m/z* 462.3 [M+H]<sup>+</sup>. Anal. Calc for C<sub>29</sub>H<sub>39</sub>N<sub>3</sub>O<sub>2</sub>: C, 75.45%; H, 8.52%; N, 9.10%. Found: C, 75.52%; H, 8.44%; N, 9.21%.

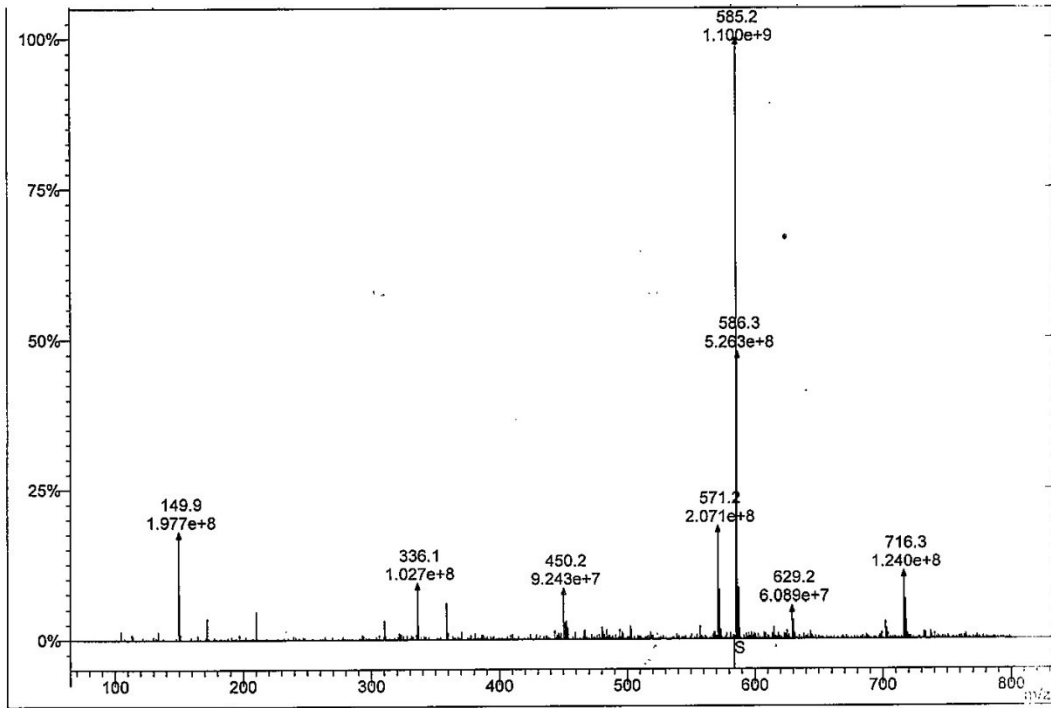


**Figure S1.**  $^1\text{H}$  NMR for **5i**.

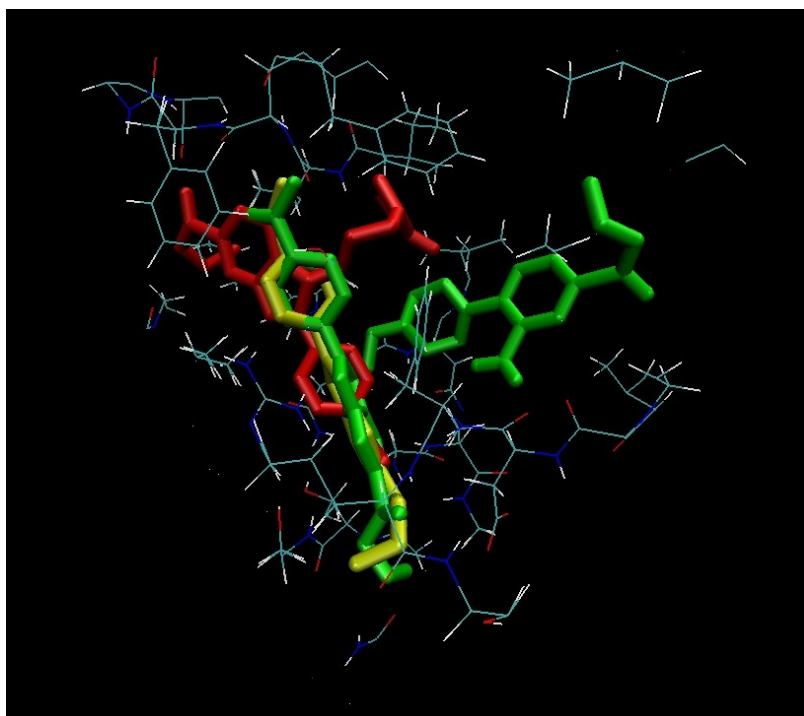


**Figure S2.**  $^{13}\text{C}$  NMR for **5i**.

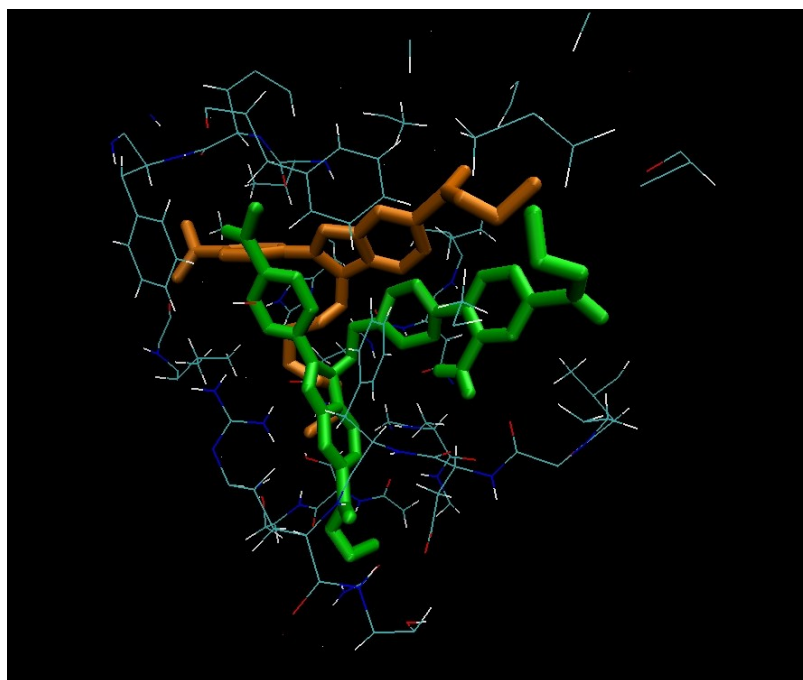




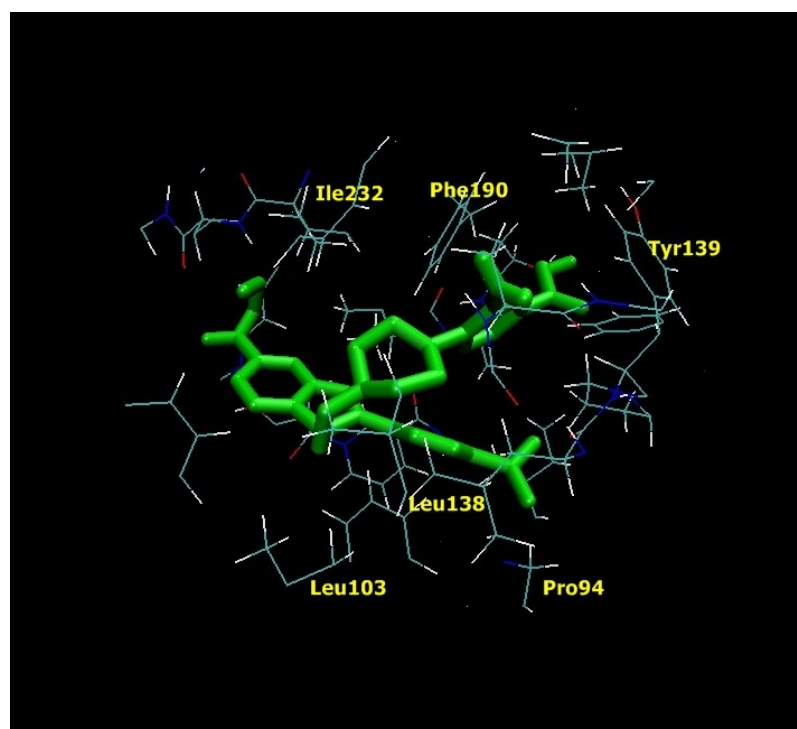
**Figure S3.** Direct Infusion MS for **5i**.



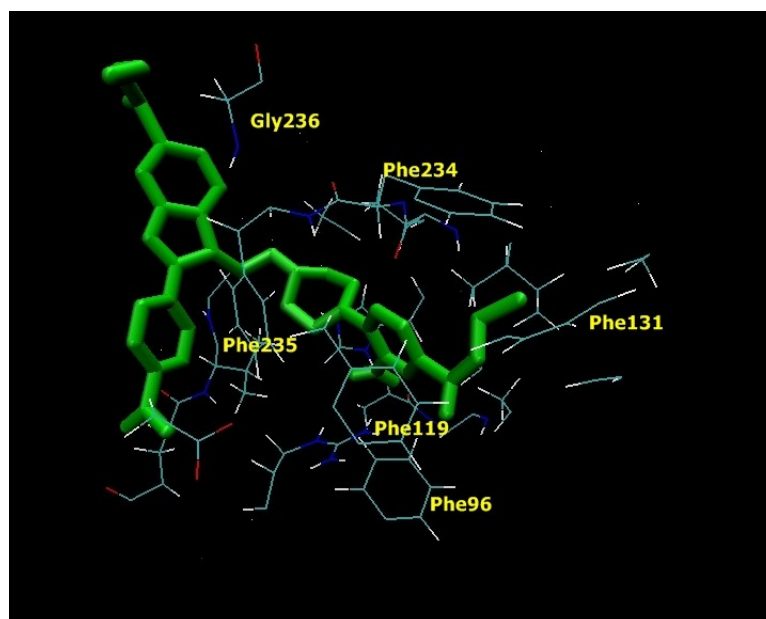
**Figure S4.** Docking of **5i** (**green**), Ethyl 2-(4-(dimethylamino)phenyl)-1H-benzo[d]imidazole-5-carboxylate (non-substituted R<sup>1</sup>; **yellow**) and ethyl 2-(4-(dimethylamino)phenyl)-1-(3-(2-oxopyrrolidin-1-yl)propyl)-1H-benzo[d]imidazole-5-carboxylate (short chain substituted R<sup>1</sup>; **red**). It was demonstrated that with shorter R<sup>1</sup> substitution, part of the docked compound was located out from the active site.



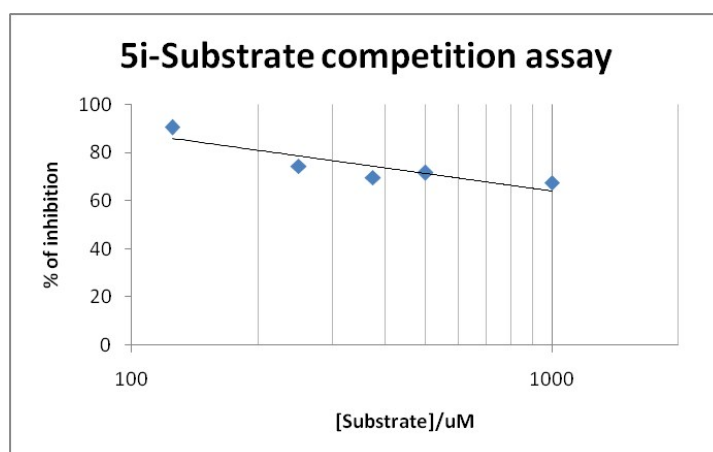
**Figure S5.** Docking pose comparison between **5i** (green) and **8i** (orange). It was found that **8i** adopts a different conformation in the SIRT2 active site ( $\sim 90^\circ$  right twist) compared to **5i**. (PDB: 3ZGV)



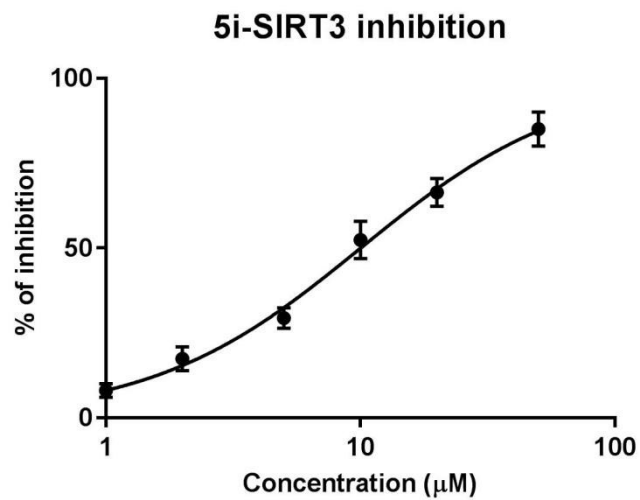
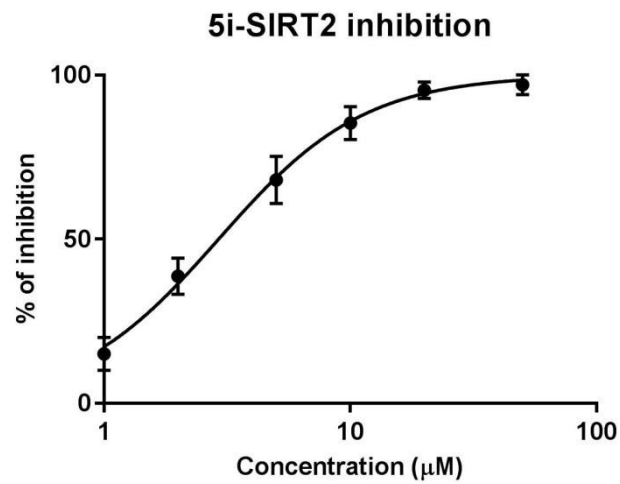
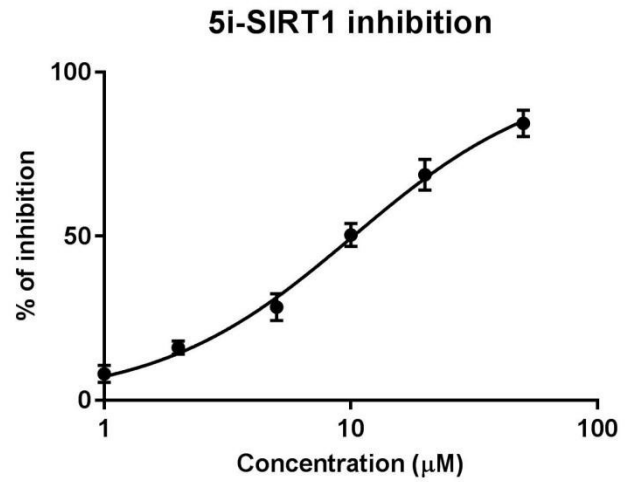
**Figure S6.** Docking pose of **5i** (green) in the extended C pocket of SIRT2 (PDB: 4RMG)



**Figure S7.** Docking pose of **5i** (green) using crystal structure PDB 5FYQ. It was found that the benzimidazole moiety was located out from the active site of SIRT2.



**Figure S8.** Competition between **5i** and SIRT2 peptide substrate at increasing concentration (125  $\mu\text{M}$ , 250  $\mu\text{M}$ , 375  $\mu\text{M}$ , 500  $\mu\text{M}$ , 1000  $\mu\text{M}$ ). Percentage of inhibition of SIRT2 plateau after 250  $\mu\text{M}$ .



**Figure S9.** SIRT1, SIRT2 and SIRT3 inhibitory plots in the presence of different concentrations of 5i. Half maximal inhibitory concentration (IC<sub>50</sub>) values were determined from these curves. Error bars showed S.D.