Supporting Information

Discovery of Potent and Selective Sirtuin 2 (SIRT2) Inhibitors Using a Fragment-based Approach

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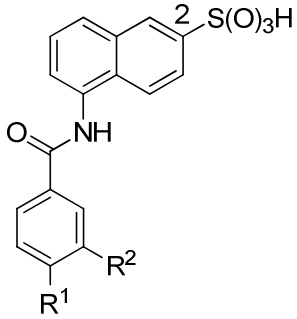

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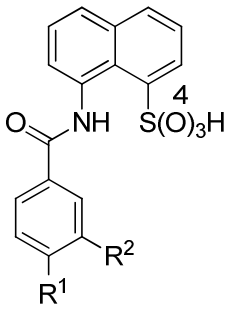

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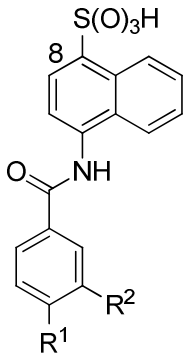

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Compound	R^1	R^2	% Inhibition at 100 μM		
			SIRT1	SIRT2	SIRT3
19	H	H	NA	NA	21 ± 6
20	Me	H	19 ± 4	24 ± 6	22 ± 10
21	H	NO_2	13 ± 7	22 ± 6	NA
22	H	NH_2	NA	NA	NA
23	Me	NO_2	19 ± 5	38 ± 4	NA
24	Me	NH_2	14 ± 4	13 ± 7	NA
25	H	H	17 ± 6	NA	15 ± 4
26	Me	H	NA	NA	10 ± 6
27	H	NO_2	NA	NA	NA
28	H	NH_2	12 ± 2	NA	NA
29	Me	NO_2	26 ± 6	NA	NA
30	Me	NH_2	12 ± 2	NA	16 ± 5
31	H	H	NA	10 ± 5	25 ± 4
32	Me	H	22 ± 7	17 ± 6	25 ± 6
33	H	NO_2	NA	29 ± 3	23 ± 6
34	H	NH_2	NA	NA	NA
35	Me	NO_2	23 ± 3	25 ± 1	22 ± 5
36	Me	NH_2	11 ± 2	13 ± 6	NA

Table S1. Inhibitory activity of the fragments based on naphthylamide sulfonic acids determined as described in the experimental subsection "SIRT1-3 Biochemical Assays". NA, < 10% inhibition. The percentages of inhibition at 100 μM were determined in duplicate and the averages were reported.

5-Benzamidonaphthalene-2-sulfonic acid (19).[1] Aniline **69** (669 mg, 3.00 mmol) was dissolved in H_2O (12 mL) and pH was adjusted to 4.8. To this stirred solution, a solution of benzoyl chloride (**72**, 588 mg, 4.20 mmol) in toluene (1 mL) was slowly added. The pH of the reaction mixture was maintained at 5.0 by addition of 2 M Na_2CO_3 solution. After the reaction was complete, the organic phase was separated and the water layer was acidified to pH = 2.0. The mixture was filtered and the filtrate was evaporated under vacuum. The resulting residue was purified by combiflash to give compound **19** as a pale solid (928 mg, 94%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 10.45 (s, 1H), 8.19 (s, 1H), 8.10 (d, J = 7.8 Hz, 2H), 7.95 (d, J = 9.0 Hz, 1H), 7.91 (d, J = 8.4 Hz, 1H), 7.73 (dd, J = 9.0, 1.8 Hz), 7.63-7.61 (m, 2H), 7.61-7.55 (m, 3H). HRMS calcd for $C_{17}H_{12}NO_4S$ $(M-H)^-$ 326.0487, found 326.0493.

5-(4-Methylbenzamido)naphthalene-2-sulfonic acid (20). In a manner similar to that was described for the preparation of compound **19**, aniline **69** (669 mg, 3.00 mmol) was treated with 4-methylbenzoyl chloride (**73**, 649 mg, 4.20 mmol) to give compound **20** as a pale solid (750 mg, 74%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 10.36 (s, 1H), 8.19 (s, 1H), 8.00 (d, J = 7.8 Hz, 2H), 7.94-7.90 (m, 2H), 7.73 (d, J = 9.0 Hz, 1H), 7.61 (d, J = 7.2 Hz, 1H), 7.55 (t, J = 7.8 Hz, 1H), 7.38 (d, J = 7.8 Hz, 2H), 2.42 (s, 3H). HRMS calcd for $C_{18}H_{14}NO_4S$ $(M-H)^-$ 340.0644, found 340.0639.

5-(3-Nitrobenzamido)naphthalene-2-sulfonic acid (21). In a manner similar to that was described for the preparation of compound **19**, aniline **69** (669 mg, 3.00 mmol) was treated with 3-nitrobenzoyl chloride (**74**, 780 mg, 4.20 mmol) to give compound **21** as a

yellow solid (1403 mg, 96%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 10.81 (s, 1H), 8.92 (s, 1H), 8.54 (d, J =7.8 Hz, 1H), 8.48 (dd, J = 7.8, 1.8 Hz, 1H), 8.21 (d, J = 1.4 Hz, 1H), 7.98-7.95 (m, 2H), 7.89 (t, J = 7.8 Hz, 1H), 7.75 (dd, J = 8.4, 1.8 Hz, 1H), 7.64 (d, J = 7.2 Hz, 1H), 7.58 (t, J = 7.8 Hz, 1H). HRMS calcd for $C_{17}H_{11}N_2O_6S$ $(M-H)^-$ 371.0338, found 371.0331.

5-(3-Aminobenzamido)naphthalene-2-sulfonic acid (22). A mixture of compound **21** (190 mg, 0.51 mmol), 10% Pd/C (20 mg) and acetic acid (one drop) in MeOH (10 mL) was hydrogenated at rt for 22 h. The reaction mixture was filtered and the filtrate was concentrated and dried in vacuo to give compound **22** as a brown solid (145 mg, 83%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 10.25 (s, 1H), 8.17 (s, 1H), 7.90 (t, J = 9.6 Hz, 2H), 7.72 (d, J = 9.0 Hz, 1H), 7.58 (d, J = 7.2 Hz, 1H), 7.54 (t, J = 7.8 Hz, 1H), 7.24-7.23 (m, 2H), 7.18 (t, J = 7.8 Hz, 1H), 6.78 (d, J = 7.8 Hz, 1H), 5.30 (s, 2H). HRMS calcd for $C_{17}H_{13}N_2O_4S$ $(M-H)^-$ 341.0596, found 341.0596.

5-(4-Methyl-3-nitrobenzamido)naphthalene-2-sulfonic acid (23). In a manner similar to that was described for the preparation of compound **19**, aniline **69** (669 mg, 3.00 mmol) was treated with 4-methyl-3-nitrobenzoyl chloride (**75**, 838 mg, 4.20 mmol) to give compound **23** as a pale solid (542 mg, 47%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 10.69 (s, 1H), 8.69 (d, J = 1.2 Hz, 1H), 8.33 (d, J = 7.8 Hz, 1H), 8.20 (d, J = 1. 8Hz, 1H), 7.95 (t, J = 9.6 Hz, 2H), 7.75-7.12 (m, 2H), 7.62 (d, J = 4.8 Hz, 1H), 7.57 (t, J = 7.8 Hz, 1H), 2.63 (s, 3H). HRMS calcd for $C_{18}H_{13}N_2O_6S$ $(M-H)^-$ 385.0494, found 385.0494.

5-(3-Amino-4-methylbenzamido)naphthalene-2-sulfonic acid (24). In a manner similar to that was described for the preparation of compound **22**, hydrogenation of compound **23** (155 mg) gave compound **24** as a brownish solid (121 mg, 85%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 10.20 (s, 1H), 8.18 (s, 1H), 7.91-7.88 (m, 2H), 7.72 (d, J = 8.4 Hz, 1H), 7.59 (d, J = 7.2 Hz, 1H), 7.54 (t, J = 7.2 Hz, 1H), 7.34 (s, 1H), 7.29 (d, J = 7.8 Hz, 1H), 7.11 (d, J = 7.8 Hz, 1H), 2.10 (s, 3H). HRMS calcd for $C_{18}H_{15}N_2O_4S$ $(M-H)^-$ 355.0753, found 355.0748.

8-Benzamidonaphthalene-1-sulfonic acid (25). In a manner similar to that was described for the preparation of compound **19**, aniline **70** (893 mg, 4.00 mmol) was treated with benzoyl chloride (**72**, 788 mg, 5.60 mmol) to give compound **25** as a pale solid (535 mg, 41%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 12.60 (s, 1H), 8.29 (dd, J = 7.2 Hz, 1H), 8.19-8.17 (m, 3H), 8.00 (d, J = 7.8 Hz, 1H), 7.80 (d, J = 7.8 Hz, 1H), 7.57-7.54 (m, 2H), 7.50 (t, J = 7.8 Hz, 2H), 7.46 (t, J = 7.8 Hz, 1H). HRMS calcd for $C_{17}H_{12}NO_4S$ $(M-H)^-$ 326.0487, found 326.0487.

8-(4-Methylbenzamido)naphthalene-1-sulfonic acid (26). In a manner similar to that was described for the preparation of compound **19**, aniline **70** (893 mg, 4.00 mmol) was treated with 4-methylbenzoyl chloride (**73**, 864 mg, 5.60 mmol) to give compound **26** as a pale solid (70 mg, 5%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 12.50 (s, 1H), 8.31 (d, J = 7.2 Hz, 1H), 8.17 (d, J = 7.8 Hz, 1H), 8.08 (d, J = 8.4 Hz, 2H), 8.00 (d, J = 7.8 Hz, 1H), 7.80 (d, J = 7.8 Hz, 1H), 7.56 (t, J = 7.8 Hz, 1H), 7.46 (t, J = 7.8 Hz, 1H), 7.31 (d, J =

7.8Hz, 1H), 2.40 (s, 3H). HRMS calcd for $C_{18}H_{14}NO_4S$ $(M-H)^-$ 340.0644, found 340.0638.

8-(3-Nitrobenzamido)naphthalene-1-sulfonic acid (27). In a manner similar to that was described for the preparation of compound **19**, aniline **70** (893 mg, 4.00 mmol) was treated with 3-nitrobenzoyl chloride (**74**, 1048 mg, 5.60 mmol) to give compound **27** as a yellowish solid (918 mg, 67%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 12.90 (s, 1H), 8.93 (s, 1H), 8.64 (d, J = 7.8 Hz, 1H), 8.42 (dd, J = 8.4, 2.4 Hz, 1H), 8.30 (d, J = 7.2Hz, 1H), 8.16 (d, J = 7.8 Hz, 1H), 8.03 (d, J =8.4 Hz, 1H), 7.87-7.83 (m, 2H), 7.60 (t, J = 7.8 Hz, 1H), 7.49 (t, J = 7.8 Hz, 1H). HRMS calcd for $C_{17}H_{11}N_2O_6S$ $(M-H)^-$ 371.0338, found 371.0344.

8-(3-Aminobenzamido)naphthalene-1-sulfonic acid (28). In a manner similar to that was described for the preparation of compound **22**, hydrogenation of compound **27** (207 mg) gave compound **28** as a brown solid (194 mg, 100%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 12.50 (s, 1H), 8.28 (d, J = 7.2 Hz, 1H), 8.10 (d, J = 7.8 Hz, 1H), 7.99 (d, J = 8.4 Hz, 1H), 7.79 (d, J = 7.8 Hz, 1H), 7.59 (d, J = 7.2 Hz, 1H), 7.55 (t, J = 7.8 Hz, 1H), 7.47-7.45 (m, 2H), 7.24 (t, J = 7.8 Hz, 1H), 6.92 (d, J = 7.2 Hz, 1H). HRMS calcd for $C_{17}H_{13}N_2O_4S$ $(M-H)^-$ 341.0596, found 341.0598.

8-(4-Methyl-3-nitrobenzamido)naphthalene-1-sulfonic acid (29). In a manner similar to that was described for the preparation of compound **19**, aniline **70** (893 mg, 4.00 mmol) was treated with 4-methy-3-nitrobenzoyl chloride (**75**, 1120 mg, 5.60 mmol) to

give compound **29** as a brownish solid (1529 mg, 99%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 12.80 (s, 1H), 8.69 (d, J = 1.8 Hz, 1H), 8.42 (dd, J = 7.8, 1.2 Hz, 1H), 8.30 (dd, J = 6.6, 1.2 Hz, 1H), 8.16 (d, J = 7.2 Hz, 1H), 8.02 (dd, J = 7.8, 1.2 Hz, 1H), 7.84 (dd, J = 7.8, 1.2 Hz, 1H), 7.67 (d, J = 8.4 Hz, 1H), 7.59 (t, J = 7.8 Hz, 1H), 7.48 (t, J = 7.2 Hz, 1H), 2.62 (s, 3H). HRMS calcd for $C_{18}H_{13}N_2O_6S$ $(M-H)^-$ 385.0494, found 385.0503.

8-(3-Amino-4-methylbenzamido)naphthalene-1-sulfonic acid (30). In a manner similar to that was described for the preparation of compound **22**, hydrogenation of compound **29** (284 mg) gave compound **30** as a brown solid (238mg, 89%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 12.40 (s, 1H), 8.27 (d, J=6.0Hz, 1H), 8.09 (d, J = 7.8 Hz, 1H), 7.97 (d, J = 7.8 Hz, 1H), 7.76 (d, J = 7.8 Hz, 1H), 7.53 (t, J = 7.8 Hz, 1H), 7.44 (t, J = 7.2 Hz, 1H), 7.36 (d, J = 7.8 Hz, 1H), 7.31 (s, 1H), 7.02 (d, J = 7.8 Hz, 1H), 2.05 (s, 3H). HRMS calcd for $C_{18}H_{15}N_2O_4S$ $(M-H)^-$ 355.0753, found 355.0757.

4-Benzamidonaphthalene-1-sulfonic acid (31).[1] In a manner similar to that was described for the preparation of compound **19**, aniline **71** (893 mg, 4.00 mmol) was treated with benzoyl chloride (**72**, 788 mg, 5.60 mmol) to give compound **31** as a pale solid (1080 mg, 83%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 10.46 (s, 1H), 8.90 (d, J = 8.4 Hz, 1H), 8.10 (d, J = 7.8 Hz, 2H), 7.98 (d, J = 7.8 Hz, 2H), 7.63 (dd, J = 7.5, 7.5 Hz, 1H), 7.58-7.50 (m, 5H). HRMS calcd for $C_{17}H_{12}NO_4S$ $(M-H)^-$ 326.0487, found 326.0492.

4-(4-Methylbenzamido)naphthalene-1-sulfonic acid (32). In a manner similar to that was described for the preparation of compound **19**, aniline **71** (893 mg, 4.00 mmol) was

treated with 4-methylbenzoyl chloride (**73**, 864 mg, 5.60 mmol) to give compound **32** as a pale solid (1342 mg, 98%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 10.35(s, 1H), 8.91 (d, J = 9.0 Hz, 1H), 8.01-7.95 (m, 4H), 7.54-7.50 (m, 3H), 7.37 (d, J = 7.8 Hz, 2H), 2.42 (s, 3H). HRMS calcd for $C_{18}H_{14}NO_4S$ $(M-H)^-$ 340.0644, found 340.0645.

4-(3-Nitrobenzamido)naphthalene-1-sulfonic acid (33). In a manner similar to that was described for the preparation of compound **19**, aniline **71** (893 mg, 4.00 mmol) was treated with 3-nitrobenzoyl chloride (**74**, 1048 mg, 5.60 mmol) to give compound **33** as a pale solid (1050 mg, 80%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 10.82 (s, 1H), 8.92 (d, J = 8.4 Hz, 2H), 8.54 (d, J = 7.8 Hz, 1H), 8.48 (dd, J = 7.8, 1.8 Hz, 1H), 8.01-7.99 (m, 2H), 7.88 (t, J = 7.8 Hz, 1H), 7.56-7.53 (m, 3H). HRMS calcd for $C_{17}H_{11}N_2O_6S$ $(M-H)^-$ 371.0338, found 371.0332.

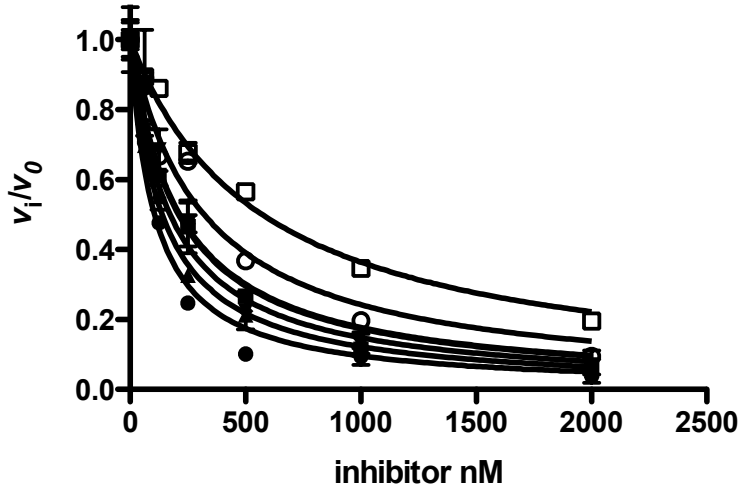
4-(3-Aminobenzamido)naphthalene-1-sulfonic acid (34). In a manner similar to that was described for the preparation of compound **22**, hydrogenation of compound **33** (170 mg) gave compound **34** as a pale solid (151 mg, 97%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 10.22 (s, 1H), 8.90 (d, J = 8.4 Hz, 1H), 7.95 (t, J = 7.8 Hz, 2H), 7.53-7.48 (m, 3H), 7.23 (t, J = 1.8 Hz, 2H), 7.17 (t, J = 8.4 Hz, 1H), 6.78 (d, J = 8.4 Hz, 1H), 5.31 (s, 2H). HRMS calcd for $C_{17}H_{13}N_2O_4S$ $(M-H)^-$ 341.0596, found 341.0588.

4-(4-Methyl-3-nitrobenzamido)naphthalene-1-sulfonic acid (35). In a manner similar to that was described for the preparation of compound **19**, aniline **71** (893 mg, 4.00 mmol) was treated with 4-methyl-3-nitrobenzoyl chloride (**75**, 1120 mg, 5.60 mmol) to

give compound **35** as a pale solid (921 mg, 60%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 10.70 (s, 1H), 8.91 (d, J = 7.8 Hz, 1H), 8.69 (s, 1H), 8.33 (d, J = 8.4 Hz, 1H), 7.98 (d, J = 7.8 Hz, 2H), 7.72 (d, J = 7.8Hz, 1H), 7.56-7.52 (m, 3H), 2.63 (s, 3H). HRMS calcd for $C_{18}H_{13}N_2O_6S$ $(M-H)^-$ 385.0494, found 385.0492.

4-(3-Amino-4-methylbenzamido)naphthalene-1-sulfonic acid (36). In a manner similar to that was described for the preparation of compound **22**, hydrogenation of compound **35** (62 mg) gave compound **36** as a yellow solid (38 mg, 67%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 10.15 (s, 1H), 8.86 (d, J = 8.4 Hz, 1H), 7.95 (t, J = 7.8 Hz, 2H), 7.53-7.48 (m, 3H), 7.29 (s, 1H), 7.23 (d, J = 7.2 Hz, 1H), 7.08 (d, J = 7.8 Hz, 1H), 2.18 (s, 3H). HRMS calcd for $C_{18}H_{15}N_2O_4S$ $(M-H)^-$ 355.0753, found 355.0753.

A



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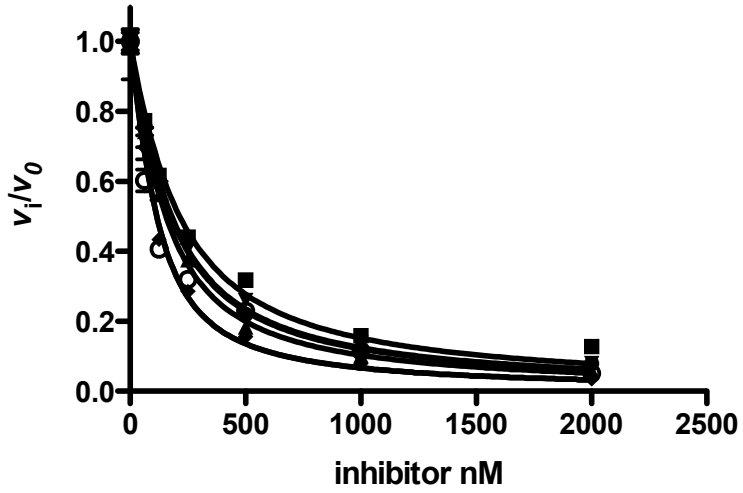

Figure S1. Inhibition of SIRT2 by **64** for the determination of its inhibition modality. Initial rate data with respect to the peptide substrate (**A**) or NAD^+ (**B**). Compound **64** was used from 0.0625 to 2.0 μM (2 fold serial dilution). **A.** The peptide substrate was used at the concentrations of 0.044 mM (●), 0.075 mM (□), 0.133 mM (▲), 0.15 mM (▼), 0.4 mM (◆), 0.6 mM (◯), and 1.2 mM(□) with NAD^+ held at 0.2 mM. **B.** NAD^+ was used at the concentrations of 0.031 mM (●), 0.063 mM (■), 0.125 mM (▲), 0.25 mM (▼), 0.5 mM (◆), and 1.0 mM (◯) with the peptide substrate held at 0.3 mM.

Reference:

(1). Jenkins, T. J.; Guan, B.; Dai, M.; Li, G.; Lightburn, T. E.; Huang, S.; Freeze, B. S.; Burdi, D. F.; Jacutin-Porte, S.; Bennett, R.; Chen, W.; Minor, C.; Ghosh, S.; Blackburn, C.; Gigstad, K. M.; Jones, M.; Kolbeck, R.; Yin, W.; Smith, S.; Cardillo, D.; Ocain, T. D.; Harriman, G. C. Design, synthesis, and evaluation of naphthalene-sulfonamide antagonists of human CCR8. *J. Med. Chem.* **2007,** *50*, 566-584.